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(54) Title: NOVEL CANNABINOID CB2 RECEPTOR AGONISTS AND USES THEREOF

(57) Abstract: Novel selective cannabinoid CB_2 receptor ligands, primarily agonists, have a number of biological and pharmacological activities, including bronchial action, immunomodulatory action and analgesia. Hence, they are useful for treating diseases or conditions characterized by pain, inflammation and immunological dysregulation. Examples of this class of novel compounds are 4 chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide and 4-chloro-*N*-[7 methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide. Other examples of the novel compounds are 4-derivatives of 3-amino and 3-carboxy-1,2-dihydro-1-substitutedquinazol-2-ones, including 2-chloro-N (1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide, 3-chloro-N-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide, tert-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate and N-benzyl-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide. The compounds bind specifically to a cell-bound or cell-free CB_2 receptor with an affinity (K_d) ≤ 100 μM. As agonists, these compounds stimulate a CB_2-related post-binding signal transduction event, e.g., inhibition of adenylyl cyclase activity, after binding to a CB_2 receptor on a cell. These compounds are used to treat inflammatory conditions, cell proliferative disorders, or an immune disorder, and may be administered in combination with agents that are also useful for the treatment of the symptoms or cause of the underlying disease or condition.

Novel Cannabinoid CB_2 Receptor Agonists and Uses Thereof

FIELD OF INVENTION

[0001] The present invention generally relates to compounds and pharmaceutical compositions comprising cannabinoid receptor agonists, processes and procedures for preparing such compounds, and to the use of these compounds in the treatment of autoimmune and inflammatory diseases and in the management of pain.

BACKGROUND

[0002] Cannabinoid receptors belong to the superfamily of G-protein coupled receptors. They are classified into the predominantly neuronal CB_1-receptors (CB_1-R) and predominantly peripheral CB_2 receptors (CB_2-R) (reviewed extensively in Howlett, AC *et al.*, *Pharmacol. Rev. 54*:161-202 (2002) which is incorporated by reference as are all the references cited therein). While the effects of CB_1-Rs are principally associated with the central nervous system (CNS), engagement of CB_2-Rs is believed to have peripheral effects related to bronchial constriction, immunomodulation and inflammation. Δ^9-tetrahydrocannabinol, the psychoactive marijuana derived cannabinoid, binds to both the CB_1-Rs and the CB_2-Rs.

[0003] It has generally been observed that compounds which stimulate the CB_2-R, agonists, suppress the immune system (Mechoulam, *Cannabinoids as Therapeutic Agents*, CRC Press, Boca Raton, Fla. (1986), Fride *et al.*, *Eur. J. Pharmacol. 231*:313 (1993), Crawley *et al.*, *Pharmacol. Behav. 46*:967 (1993) and Smith *et al.*, *J. Pharm. Exp. Therap. 270*:219 (1994)).

[0004] The pharmacology and therapeutic potential of ligands of the cannabinoid receptor system have been reviewed (Howlett *et al.*, *supra*, *Exp. Opin. Ther. Patents* 1998, *8*, 301-313; A. Doherty, Ed., *Ann. Rep. Med. Chem.*, *34*: 199-208 (1999); *Exp. Opin. Ther. Patents* 2000, *10*:1529-1538; *Trends in Pharm. Sci.* 2000, *21*:218-224).

Cannabinoids: Classical and Nonclassical Agonists and Antagonists

Classical Cannabinoids

[0005] Classical cannabinoids consist of ABC-tricyclic dibenzopyran derivatives that are either compounds occurring naturally in the plant, *Cannabis sativa*, or synthetic analogs of these compounds such as Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-hydroxydimethylheptyl (HU-210), and desacetyl-L-nantradol. Of these, Δ^9-THC is the main psychotropic constituent of cannabis is also a psychotropic plant cannabinoid, whereas HU-210 and desacetyl-L-nantradol are synthetic cannabinoids. All these cannabinoids elicit cannabimimetic responses both *in vivo* and *in vitro*. Δ^9-THC, first isolated from *C. sativa* has an absolute stereochemistry of (*6a*R,*10a*R). Δ^9-THC undergoes significant binding to cannabinoid receptors at submicromolar concentrations, with similar affinities for CB_1 and CB_2-Rs. At CB_1-Rs, it behaves as a partial agonist, the size of its maximal effect falling below that of synthetic cannabinoid receptor agonists with higher relative intrinsic activity, such as CP55940 and R-(+)-WIN55212. The relative intrinsic activity of Δ^9-THC at CB_2-Rs is even less than at CB_1-Rs. Indeed Δ^9-THC behaved as a CB_2-R antagonist when incubated with CHO cells transfected with human CB_2-Rs ("hCB_2-R") using a cyclic AMP (cAMP) assay (Bayewitch *et al.*, *J Biol Chem* **271:**9902–9905, 1996).

[0006] Δ^8-THC has affinities for CB_1 and CB_2-Rs that are similar to those of Δ^9-THC, though its synthetic analog, HU-210, has relative intrinsic activities at CB_2-Rs that match those of the high-efficacy agonists, CP55940 and (+)-WIN55212 and even higher affinity, making it a particularly potent cannabinoid receptor agonist. Its pharmacological effects *in vivo* are also exceptionally long lasting. The enhanced affinity and relative intrinsic activity of HU-210 can be largely attributed to the replacement of the pentyl side chain of Δ^8-THC with a dimethylheptyl group.

[0007] Most classical cannabinoids that bind to CB_1 have affinity for CB_2 as well, without major selectivity for either of these receptors (similar Ki values but significantly differential levels of potency). For example, Δ^9-THC has a Ki of about 40 nM for either receptor; the Ki of HU-210 is about 100 times lower (*i.e.*, higher affinity) (Showalter *et al.*, *J Pharmacol Exp Ther* **278:**989–999, 1996).

[0008] Table 1 below shows CB_2-selective ligands in order of increasing CB_2/CB_1 selectivity.

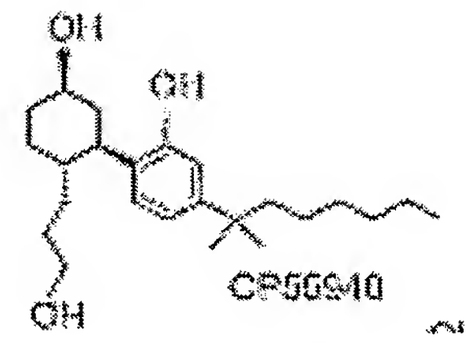
[0009] Stereochemical changes can also affect the pharmacological activity of cannabinoids of the cannabidiol-type. (+)-CBD, (+)-5'-dimethylheptyl-CBD, and (+)-7-OH-5'-dimethylheptyl-CBD each has greater affinity for CB_2-Rs than its corresponding (-)-enantiomer. However, the stereochemical prerequisites for binding to CB_2-Rs are not the same in the cannabidiol series ((+)(*3*S,*4*S) enantiomers have greater affinity) as in the THC series ((-)(6*a*R,10*a*R) enantiomers have greater affinity).

[0010] CB_2-selective agonists were developed from the first generation of classical cannabinoids by making relatively minor changes to the THC molecule. Removal of the phenolic OH group from HU-210 to form 1-deoxy-11-OH-Δ^8-THC-dimethyl-heptyl (JWH-051) greatly enhanced affinity for CB_2-Rs without significantly affecting CB_1 affinity.

[0011] The high degree of CB_2 selectivity (by binding) of JWH-133, JWH-139, and HU-308 and by the Merck Frosst compounds L-759633 and L-759656 (Merck Frosst Canada Ltd.) are remarkable as all of these bind to CB_2-Rs at concentrations in the low nanomolar range. The latter two compounds are equally potent and efficacious with CP55-940, the high relative intrinsic activity agonist, at inhibiting forskolin-stimulated cAMP accumulation in CHO cells expressing recombinant CB_2-Rs (Ross *et al.*, *Br J Pharmacol* **132:**631–640, 1999). Similarly, HU-308 and JWH-133 are much more potent inhibitors of forskolin-stimulated cAMP production by CB_2-transfected than by CB_1-transfected CHO cells.

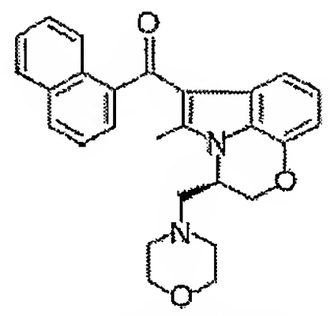
Nonclassical Cannabinoids

[0012] Scientists at Pfizer synthesized new analogs lacking the dihydropyran ring of THC. (Melvin *et al.*, *J Med Chem* **27:**67–71, 1984; Melvin *et al.*, 1993 *Mol Pharmacol* **44:**1008– 1015). Further developments ultimately led to the bicyclic analog, CP55940, which has become one of the most widely studied cannabinoid agonists.

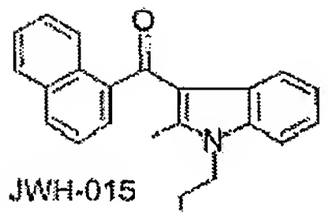


[0013] Use of [^{3}H]-CP55940 (less lipophilic than THC)) allowed the discovery and characterization of the CB_1 cannabinoid receptor; this ligand is still the most used radiolabeled cannabinoid ligand as it binds to CB_1 and CB_2-Rs with similar affinity and displays high activity *in vivo* as well, being 10 to 50 times more potent than Δ^9-THC. CP55940 behaves as a full agonist for both receptor types, and its maximal effects in CB_2-R assay systems equal or exceed those of other cannabinoid receptor agonists. Like classical cannabinoids, nonclassical cannabinoids with chiral centers exhibit significant stereoselectivity, those compounds with the same absolute stereochemistry as (-)-Δ^9-THC at 6*a* and 10*a* (6*a*R,10*a*R) exhibit the greater pharmacological activity.

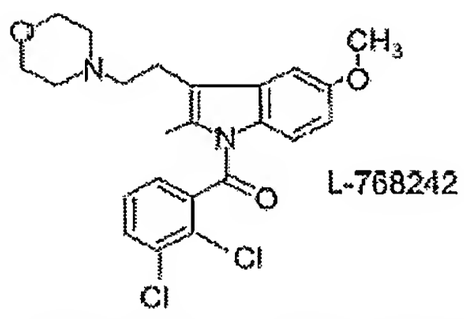
[0014] In the 1990's Sterling Winthrop researchers reported a new family of aminoalkylindoles with cannabimimetic properties. R-(+)-WIN55212 (see structure below), the best studied commercially available compound of the series, displays high affinity for both receptor types, with moderate selectivity in favor of CB_2-R and high relative intrinsic activity at both (Bouaboula *et al.*, *J Biol Chem* **272:**22330–22339, 1997)). *In vivo*, it produces the full spectrum of THC's pharmacological effects and substitutes for other cannabinoids in behavioral tests. In contrast, its S-(-)-enantiomer, R-(+)WIN55212-3, lacks activity both *in vivo* and *in vitro* (Pertwee, *Curr Med Chem* **6:**635–664, 1999). A tritiated form of this ligand, [^{3}H]R-(+)-WIN55212, was also prepared.



R-(+)-WIN55212



JWH-015



L-768242

[0015] Other aminoalkylindole derivative cannabinoid receptor agonists have been prepared (see Huffman, *1999 Symposium on the Cannabinoids*; 1999 Jun 18–20; Acapulco, Mexico. pp 10, International Cannabinoid Research Society, Burlington, VT), making it possible to show that activity is retained when the aminoalkyl substituent is replaced by simple n-alkyl chains or when the indole nucleus is replaced by a pyrrole or indene ring. Some of these newer aminoalkylindoles displayed significant selectivity for the CB_2-R, namely JWH-015 (above) and a series of Merck Frosst compounds that includes L-768242 (above).

TABLE 1

CB_2-selective ligands in order of increasing CB_2/CB_1 selectivity[a]		
Ligand	**CB_1 *Ki* Value (*nM*)**	**CB_2 *Ki* Value (*nM*)**
JWH-015	383	13.8
JWH-051	1.2[b]	0.032
L-768242	1,917	12
JWH-139	2,290[b]	14
AM 630	5,152	31.2
JWH-133	677[b]	3.4
L-759633	1,043	6.4
	15,850	20
L-759656	4,888	11.8
	>20,000	19.4
HU-308	>10,000[b]	22.7
SR144528	437	0.60
	05[b]	0.30[b]
	10,000	5.6
Cannabinoid receptor ligands without marked CB_2/CB_1 selectivity		
Ligand	**CB_1 *Ki* Value (*nM*)**	**CB_2 *Ki* Value (*nM*)**
Anandamide	61[a,b]	1,930[a,c]
	89[a]	371[a]
	543	1,940
	71.7[a,b]	279[a,b]
	252[b]	581
2-Arachidonoylglycerol	472[b]	1,400
	58.3[d]	145[d]
HU-210	0.0608	0.524
	0.1[b]	0.17
	0.73	0.22
CP55940	5	1.8
	3.72	2.55
	1.37[b]	1.37[b]
	0.58	0.69
	0.50[a,b]	2.80[a,b]
Δ^9-THC	53.3	75.3
	39.5[b]	40
	40.7	36.4
	80.3[b]	32.2
	35.3[b]	3.9[b]
Δ^8-THC	47.6[b]	39.3[c]
R-(+)-WIN55212	9.94[b]	16.2[b]
	4.4[a,b]	1.2[a,b]
	1.89	0.28
	62.3	3.3
	123	4.1

a From Howlett *et al., supra); b* Binding to rat cannabinoid receptors on transfected cells or on brain (CB_1) or spleen tissue (CB_2). *c* Binding to mouse spleen cannabinoid receptors. *d* Species unspecified. All other data from experiments with human cannabinoid receptors.

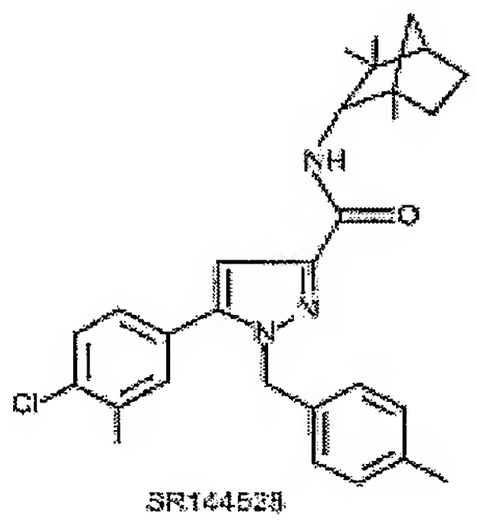
Eicosanoids

[0016] The prototypic member of the eicosanoid group of cannabinoid receptor agonists is anandamide, which belongs to the 20:4, n-6 series of fatty acid amides. This was the first of five endogenous agonists to have been discovered in mammalian brain and other tissues (Devane *et al.*, *Science 258*:1946–1949, 1992), which along with 2-arachidonoylglycerol (Mechoulam *et al.*, *Biochem Pharmacol* **50:**83–90, 1995) are the best studied of this class.

[0017] Anandamide is a partial agonist at CB_1-Rs and exhibits less relative intrinsic activity at CB_2 than CB_1-Rs. 2-Arachidonoylglycerol is an agonist for both CB_1 and CB_2-Rs and exhibits higher relative intrinsic activity than anandamide at both. 1(3)-arachidonoylglycerol has similar CB_1 and CB_2 binding properties.

Antagonists/Inverse Agonists

[0018] A prototypic member of the diarylpyrazoles series of compounds is the Sanofi compound SR144528, a potent CB_2 -selective ligand.



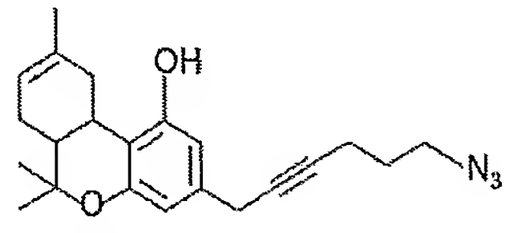
SR144528

These ligands prevent or reverse effects mediated by CB_2-Rs. By itself, SR144528 can act on CB_2-Rs to produce effects that are opposite to those produced by agonists (Pertwee, 1999, *supra*). SR144528 can evoke inverse agonist responses, based on the ability of CB_2-Rs to exhibit signal transduction constitutively. As such, arylpyrazoles reduce the constitutive activity of these signalling pathways.

[0019] AM630 is a CB_2-selective antagonist/inverse agonist that is potent (though less than SR144528) in reversing CP55940-induced inhibition of forskolin-stimulated cAMP production in hCB_2-R-transfected CHO cells and, when administered alone, enhances forskolin-stimulated cAMP production and

inhibits [^{35}S]GTPγS binding (Ross *et al.*, *supra*). AM630 has mixed agonist-antagonist properties and is a low-affinity partial CB_1-R agonist (Pertwee *supra*).

[0020] One compound that is close to being a pure CB_1/CB_2-R antagonist (that lacks any agonist or inverse agonist activity) is the classical cannabinoid 6'-azidohex-2'-yne-Δ^8-THC (O-1184). In addition to a terminal N_3 group, the C-3



O-1184

alkyl side chain includes a C≡C (fix) triple bond, which decreases relative intrinsic activity at both receptors without affecting affinity (Ross *et al.*, *Br J Pharmacol* **132:**631–640, 1999). At CB_2-Rs, O-1184 behaves as a high-affinity, low-efficacy inverse agonist; replacement of the triple bond with a C=C double bond, results in a compound which acts as a high-affinity, low-efficacy partial agonist at CB_2-Rs.

Molecular Biology of Cannabinoid Receptors

[0021] Although the existence of cannabinoid receptors was known before their cloning, the receptors presently known as CB_1 and CB_2 cannabinoid receptors were cloned as part of strategies based on conserved sequence motifs to clone G protein-coupled receptors (GPCR) in general rather than specifically trying to clone cannabinoid receptors. It was only after extensive screening of an expressed rat brain cDNA clone that it was identified as the CB_1 cannabinoid receptor (Matsuda *et al.*, *Nature 346*:561–564, 1990; see Matsuda, *Crit Rev Neurobiol* **11:**143–166, 1997, for review). Human and mouse homologues have since been reported. They encode proteins of 472 (human) or 473 (rat, mouse) amino acids, including a rather long and well conserved amino terminal extracellular domain of 116 to 117 residues. Overall, these three receptors have 97 to 99% amino acid sequence identity. There was no substantial evidence for a second cannabinoid receptor until hCB_2 cDNA was cloned from HL-60 cells (Munro *et al.*, *Nature 365*:61–65, 1993). Its 360-amino acid sequence is quite different from that of CB_1, especially in its much shorter amino terminal domain with no significant conservation. Between trans-membrane domains 1 and 7, the

CB_2 protein is only 48% identical to the CB_1 protein, substantially less than the 70 to 80% usually seen between different types of GPCRs. It is reported to be expressed primarily in spleen. The mouse CB_2 gene has been cloned (Shire *et al.*, *Biochim Biophys Acta 1307*:132–136, 1996) and is only 82% identical at the protein level to the human receptor and 13 amino acids shorter at the C-terminus. The rat gene is similar to the mouse gene, but is 13 residues longer at the C-terminus. The coding sequence is contained in a single exon of the mouse gene (see GenBank accession no. U21681), but available cDNA sequence indicates that there is at least one additional exon containing only 5'-untranslated sequence.

[0022] Site-directed mutagenesis has recently begun to define which residues constitute the agonist binding sites. Lys192 is located at the extracellular end of helix three in both the CB_1 and CB_2-Rs, a region commonly implicated in agonist binding in other GPCRs. Conservative substitution of an Arg for the Lys had little effect, whereas the potentially disruptive substitutions of Gln or Glu eliminated binding of CP55940 (but had little effect on binding of R-(+)-WIN55212. Mutations of the hCB_2-R at Lys109 to Arg or Ala had little effect. Molecular modeling of the two Ala- substituted receptors (CB_1 K192A and CB_2 K109A) indicated that CB_2-R still could bind its ligand via hydrogen bonding to Ser112 that were absent in CB_1 at the corresponding residue, Gly195. When the CB_2 K109A receptor was further modified to convert Ser112 to Gly, it acted only on the CB_1 K192A mutant receptor, confirming the modeling prediction. There appear to be two adjacent residues on the same face of helix 3 which play a critical role in binding of agonists (other than R-(+)-WIN55212). Other mutations altering residues that are highly conserved throughout the rhodopsin family of GPCRs, such as the Asp in helix 2, the DRY motif at the intracellular end of helix 3, the Trp in the middle of helix 4 and the Tyr near the intracellular end of helix 7, result in the same types of effects as analogous mutations in other receptors. Given the highly conserved nature of these residues and their positions generally near the intracellular ends of the respective helices, it is likely that they are less relevant for agonist binding but rather important for conformations needed for transmitting a binding signal to the G protein. More relevant to the agonist binding sites is the Trp at the extracellular end of helix 4. Conservative mutations

of Trp172 in hCB_2 to Phe and Tyr had little effect, but removal of the aromatic side chain by substitution of Ala or Leu eliminated ligand binding.

Tissue Distribution of Cannabinoid Receptors

[0023] CB_2-Rs are primarily localized on cells in structures associated with the immune system and when analyzed on dissociated cells, are primarily on mature B cells, and, to a lesser degree, macrophages. In addition to their CNS localization, CB_1-Rs are widely expressed in the peripheral nervous system (sensory nerve fibers and autonomic nervous system (e.g., Pertwee *et al.*, *Br J Pharmacol 105*:980–984, 1992). CB_1-Rs are also expressed in some immune cells, but their level of expression is considerably lower than that of CB_2-Rs

Distribution of Cannabinoid Receptors in Immune System

[0024] Current knowledge about the distribution of CB_1 and CB_2 cannabinoid receptors in cells and tissues of the immune system is summarized in Table 2 (see Howlett *et al.*, *supra*, for details and individual references).

TABLE 2: ***Detection of cannabinoid receptors in immune cells and tissues***

Cell Type/Tissue	Species	Receptor	Method of Detection
B lymphocytes	Human	CB_2	RT-PCR[a] or confocal microscopy[b]
Macrophages	Human, mouse, rat	CB_2	RT-PCR[a,c,d]
Mast cells	Rat	CB_2	RT-PCR[e]
Microglia	Rat	CB_1 ,CB_2	Mutational RT-PCR[d,f] Western immunoblot[d,f] or immunocytochemistry[f,g]
Natural killer cells	Human	CB_2	RT-PCR[a]
Peripheral blood mononuclear cells	Human, rat	CB_2	RT-PCR[a,e]
CD4 lymphocytes	Human	CB_2	RT-PCR[a]
CD8 lymphocytes	Human	CB_2	RT-PCR[a]
Lymph nodes	Human	CB_2	RT-PCR[a]
Peyer's patches	Rat	CB_1 and/or CB_2	Radioligand binding[h] or radioligand autoradiography[h]
Spleen	Human, mouse, rat	CB_1, CB_2	Radioligand binding[h,i] radioligand autoradiography[h] Northern blot[j] *in situ* hybridization[j] or RT-PCR[a,e]
Tonsils	Human	CB_2	RT-PCR[a] or immunocytochemistry[a]
Thymus	Human	CB_2	RT-PCR[a]

a Galiegue *et al.*, 1995; *b* Carayon *et al.*, 1998; *c* Lee *et al.*, 2001; *d* Carlisle *et al.*, 2002; *e* Facci *et al.*, 1995;
f Waksman *et al.*, 1999; *g* Sinha *et al.*, 1998; *h* Lynn and Herkenham, 1994; *i* Kaminski *et al.*, 1992; *j* Munro *et al.*, 1993.

[0025] CB_1-R mRNA is found primarily in neural tissue, and in peripheral tissues, including the adrenal gland, bone marrow, heart, lung, prostate, testis, thymus, tonsils, and spleen. CB_1-R mRNA has also been found in select immune cell lines, including human THP-1 monocytic cells, human Raji B-cells, murine NKB61A2 natural killer ("NK")-like cells, and murine CTLL2 IL-2-dependent T cells (Daaka *et al.*, In *The Brain Immune Axis and Substance Abuse* (Sharp BM *et al.*, eds, Plenum Press, New York, 1995, pp 91–96).

[0026] Both *in situ* hybridization and autoradiographic studies suggested expression of CB_2-Rs in multiple lymphoid organs (Lynn *et al.*, *J Pharmacol Exp Ther 268:*1612–1623, 1994; Buckley *et al.*, *Neuroscience 82:*1131–1149, 1998). CB_2-R mRNA is found in spleen, thymus, tonsils, bone marrow, pancreas, splenic macrophage/monocyte preparations, mast cells, peripheral blood leukocytes, and in a variety of cultured immune cell models, including the myeloid cell line U937 and undifferentiated and differentiated granulocyte-like or macrophage-like HL-60 cells (Bouaboula *et al.*, *Eur J Biochem* **214:**173–180, 1993; Condie *et al.*, *J Biol Chem 271*:13175– 13183, 1996; Schatz *et al.*, *Toxicol Appl Pharmacol 142:*278–2871997). Valk *et al.* (*Blood 90*:1448–1457, 1997) reported the presence of CB_2-R mRNA in 45 of 51 cell lines of distinct hematopoietic lineages, including myeloid, macrophage, mast, B-lymphoid, T-lymphoid, and erythroid cells. In spleen and tonsils, CB_2 mRNA content is equivalent to that of CB_1 mRNA in the CNS. However, the distribution pattern of CB_2 mRNA displays major variation in human blood cell populations, with a rank order of B lymphocytes > NK cells >> monocytes > polymorphonuclear neutrophils > CD8 lymphocytes > CD4 lymphocytes (Galiegue *et al.*, *Eur J Biochem* **232:**54–61, 1995). A rank order for CB_2 mRNA content similar to that noted for primary human cell types has been recorded for human cell lines belonging to the myeloid, monocytic, and lymphoid lineages. The pattern of distribution in murine immune cell subpopulations is similar (Lee *et al., Eur J Pharmacol* **423:**235–241, 2001) with CB_2 mRNA most abundant in splenic B cells, followed by macrophages and T cells. Cannabinoid receptor protein has also been localized in a variety of immune cell types and tissues using ligand binding assays (Lynn *et al.*, *supra*). Cannabinoid receptor binding was limited to B cell-enriched areas (marginal zone of spleen, cortex of lymph nodes, and nodular corona of Peyer's patches), whereas

specific binding was absent in T cell-enriched areas (thymus, periarteriolar lymphatic sheaths of the spleen), and certain macrophage-enriched areas (liver and lung). Bouaboula *et al.*, 1993, *supra* used [^{3}H]CP55940 as a ligand for characterizing cannabinoid receptors in membranes of human myelomonocytic U937 cells. A Kd of 0.1 nM and a Bmax of 525 fmol/mg protein was determined from Scatchard analysis.

[0027] CB_1- and CB_2-specific antibodies have been used to identify cannabinoid receptors in immune cells. CB_1-R protein has been identified in the human Jurkat T cell line (Daaka *et al.*, *J Pharmacol Exp Ther* **276:**776–783, 1996), in Daudi human B-lymphoblastoid cells and macrophage-like cells from rat brain tissue, and in cortical microglia cultured from neonatal rat brain (Waksman *et al.*, *J Pharmacol Exp Ther* **288:**1357–1366. Galiegue *et al.*, 1995, *supra* used an anti-hCB_2 IgG to localize CB_2-Rs within B lymphocyte-enriched areas of the mantle of secondary lymphoid follicles in sections of human tonsil. Carayon *et al.* (*Blood* **92:**3605–3615, 1998) studied expression of CB_2-Rs in leukocytes using purified polyclonal antibodies and found that peripheral blood and tonsillar B cells expressed the highest levels of CB_2-R protein.

[0028] Changes in levels of cannabinoid receptors or their mRNAs occur after treatment with a variety of immune modulators or activators. CB_2 mRNA was present in thioglycollate-elicited murine peritoneal macrophages but not in resident peritoneal macrophages. CB_2 mRNA expression was studied following immune cell "activation." Bacterial lipopolysaccharide (LPS) down-regulated CB_2 mRNA expression in spleen cell cultures in a dose-responsive manner, whereas stimulation through CD40 using anti-CD40 antibody had an upregulating effect, which was attenuated by costimulation with IL-4. LPS-stimulated Raji cells and PMA-stimulated THP1 human acute monocytic leukemia cell lines had increased levels of CB_1 cannabinoid receptor mRNA linked to comparable increases in receptor protein expression. Mitogen activation of Jurkat cells caused increased specific binding of [^{3}H]CP55940; Western analysis indicated the presence of the receptor protein on membranes from mitogen-activated, but not unstimulated, Jurkat T cells. Anti-CD40, anti-CD3, and IL-2 stimulation induced contrasting changes in CB_1 mRNA expression in mouse spleen cells; stimulation with T cell mitogens PMA/Ionomycin and anti-CD3 resulted in lower CB_1-R

message, whereas stimulation with the B-cell mitogen anti-CD40 increased message levels. Treatment with a combination of mitogens and IL-2 uniformly raised CB_1-R mRNA levels (Noe *et al.*, *J Neuroimmunol* **110:**161–167, 2000). The foregoing suggests that signaling pathways activated by T cell mitogens lead to decreased CB_1 gene activation, whereas pathways activated by B-cell mitogens and IL-2 lead to increased CB_1 gene activation. Collectively, these findings suggest that cannabinoid receptors are of biological relevance in lymphoid and myeloid cells during discrete stages of activation. Changes in levels of rat spleen cannabinoid receptors have been reported after chronic cannabinoid (CP55940) administration *in vivo* (Massi *et al.* , *Pharmacol Biochem Behav 58*:73–78, 1997) which led to significant loss of [^{3}H]CP55940 binding in chronic treated, tolerant rats as measured by densitometry and autoradiography of spleen sections.

Functional Immunological Effects

[0029] The presence of peripheral cannabinoid receptor mRNA and protein in a variety of immune cell types, and the recognition that cannabinoids inhibit adenylyl cyclase in immune cells through a pertussis toxin-sensitive mode, support a role for cannabinoid receptors in the modulation of immune cell functions (Kaminski and colleagues, *Mol Pharmacol 42*:736– 742, 1992; *Biochem Pharmacol 48*:1899–1908; *Toxicol Lett 102–103*:59 –63). Suppression of antibody responses by cannabinoids was mediated in part through inhibition of adenylyl cyclase by a pertussis toxin-sensitive G protein-coupled mechanism. Δ^9-THC and the synthetic agonist CP55940 inhibited the lymphocyte proliferative and the sheep erythrocyte IgM antibody-forming cell responses of murine spleen cells to PMA/ionomycin. More direct evidence for a functional linkage came from the use of CB_1- and CB_2-selective antagonists. Select activities of macrophages are affected by cannabinoids through cannabinoid receptors.

[0030] Δ^9-THC modulated the antigen processing capacity by macrophages that is necessary for activation of CD4+ T lymphocytes. Inhibition of the processing of a protein antigen was blocked by the CB_2–R-selective antagonist, SR144528, whereas the CB_1-R-selective antagonist SR141716A had no such effect (McCoy *et al., J Pharmacol Exp Ther* **289:**1620–1625, 1999). These observations were confirmed using CB_2-R knockout mice (Buckley *et al.*, 2000) in which Δ^9-THC inhibited helper T cell activation by macrophages in wild

type, but not knockout, mice, consistent with a role for the CB_2-R in antigen processing. Sacerdote *et al.*, *J Neuroimmunol* **109:**155–163, 2000 reported that *in vivo* and *in vitro* treatment with the synthetic cannabinoid CP55940 decreased *in vitro* migration of rat macrophages, involving both CB_1 and CB_2-Rs. Both spontaneous cell migration, and that induced by a chemotactic peptide, were affected. While both SR141716A and SR144528 blocked the CP55940-induced inhibition of spontaneous migration, the CB_2 antagonist was more potent, and only it reversed the effect on induced chemotaxis. The endocannabinoid 2-arachidonoylglycerol stimulated constitutive nitric oxide release from human monocytes and vascular tissues, mediated through the CB_1-R. In both the monocytes and lymphocytes, NO release elicited in response to 2-arachidonoylglycerol exposure was blocked by a CB_1 antagonist but not by a CB_2 antagonist (Stefano *et al., Pharmacol Res* **42:**317–322, 2000).

[0031] CB_1 and CB_2-Rs appear to be involved in Δ^9-THC-induced inhibition of NK cell activity (Massi *et al.*, *Eur J Pharmacol* **387:**343–347, 2000). *In vivo* administration of Δ^9-THC to mice significantly inhibited NK cytolytic activity without affecting concanavalin A (Con A)-induced T cell proliferation. Pretreatment with the CB_1 and CB_2 cannabinoid receptor antagonists partially reversed this inhibition with the CB_1-R antagonist being more effective than the CB_2-R antagonist. Parallel measurements of interferon γ (IFN-γ) revealed that Δ^9-THC significantly reduced production of this cytokine. Antagonists at both receptors completely reversed the IFNγ reduction. Thus, both receptor types are involved in the complex network regulating and mediating NK cytolytic activity.

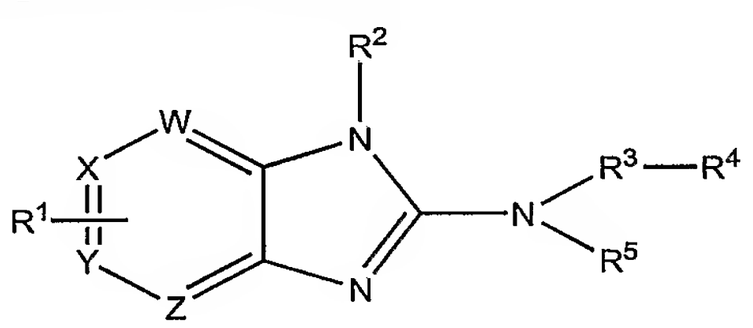
[0032] Carayon *et al.*, *supra* reported downregulation of CB_2-R expression (mRNA and protein) during B-cell differentiation with lowest expression in germinal center proliferating centroblasts of tonsils. The nonselective agonist CP55940 enhanced CD40-mediated proliferation of both virgin and germinal center B-cell subsets, and this was blocked by the CB_2-R-selective, but not the CB_1-R-selective antagonist. CB_2-Rs were up-regulated in both B-cell subsets during the first 24 h of CD40-induced activation. It therefore appears that CB_2 cannabinoid receptors play a role in B cell differentiation.

[0033] To explain how cannabinoids, acting through cannabinoid receptors, exert such a broad spectrum of effects on immune function, one may postulate that they act through differential expression of cytokines. Δ^9-THC and other cannabinoid agonists stimulate expression of "immune inhibitory" Th2-type cytokines while inhibiting Th1-type "immune stimulatory" cytokines. Δ^9-THC inhibited antitumor immunity against lung tumors of differing immunogenicity by a CB_2-R-mediated, cytokine-dependent pathway (Zhu *et al.*, *J Immunol* **165:**373–380, 2000). Δ^9-THC appeared to decrease tumor immunogenicity, as reflected in the lower capacity of tumor-immunized, Δ^9-THC-treated mice to withstand challenge. The Th2 cytokines, IL-10 and TGFβ were augmented in these animals, whereas the Th1 cytokine, IFN-γ, was suppressed, both at the tumor site and in the spleen. *In vivo* treatment with a CB_2-selective antagonist (SR144528) blocked the THC effect, suggesting that a CB_2-R-mediated, cytokine-dependent pathway was responsible for Δ^9-THC promoting tumor growth by inhibiting antitumor immunity. Δ^9-THC treatment of BALB/c mice also suppressed immunity and early IFN-γ, IL-12, and IL-12 receptor β2 responses to *Legionella pneumophila*. Levels of Th1 cytokines IL-12 and IFN-γ, as well as resistance to a challenge infection, were suppressed. Results with selective antagonists indicated that both the CB_1 and CB_2-Rs were involved.

[0034] There is thus a need in the art to develop improved cannabinoid receptor ligands that can be harnessed to inhibit undesirable immune response, such as those seen in autoimmune and allergic disease, and to inhibit the inflammatory sequelae of the immunological processes in this setting. Embodiments of the invention are directed to this goal, and relate to novel compounds that act on CB_2-R.

SUMMARY

[0035] To address this need, novel selective CB_2-R ligands, primarily agonists, were developed. These compounds have a number of biological and pharmacological activities, including bronchial constriction, anti-inflammatory action, immunomodulatory action and analgesia. Hence, they are useful for treating diseases or conditions characterized by pain, inflammation and immunological dysregulation. More specifically, the embodiments of the invention relate to compounds of Formula I:



Formula I

prodrugs thereof, or pharmaceutically acceptable salts, solvates or stereoisomers of the compounds or of the prodrugs. In the preferred compounds of this embodiment:

R^1 is: H, $C_{1\text{-}6}$ alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or $C_{2\text{-}6}$ alkoxy.

R^2 is: $C_{1\text{-}6}$ alkyl, cycloalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl wherein n is an integer from 1 to 3.

R^3 is: CHR^6, CO or SO_2.

R^4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl.

R^5 is: H or lower alkyl or heteroalkyl.

R^6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy.

W, X, Y and Z can be either C or N, wherein the total number of N atoms amongst W, X, Y and Z does not exceed 2.

[0036] Preferred embodiments of the compounds of Formula I are:

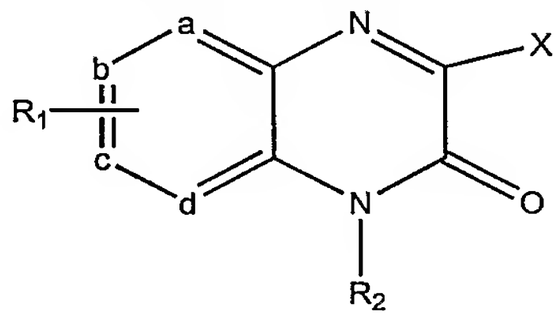
(a) 4-chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide;

(b) 4-chloro-*N*-[7-methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide;

(c) 3,4-dichloro-N-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazo]benzamide; and

(d) 3,4-dichloro-N-(1-(2-morpholinopropyl)-1*H*-benzo[d]imidazol-2-yl)benzamide.

[0037] Also described herein are compounds of Formula II:



Formula II

In the preferred compounds of this embodiment:

R_1 is: H, C_{1-6} alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or C_{2-6} alkoxy.

R_2 is: C_{1-6} alkyl, cycloalkyl, $(CH_2)_n$-heteroalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl wherein n is an integer from 1 to 3.

X is: $N(R_3\text{-}R_4)(R_5)$, C(O)Y or C(NH)Y.

R_3 is: CHR_6, CO or SO_2.

R_4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl.

R_5 is: H or lower alkyl or heteroalkyl.

R_6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy.

Y is: $N(R_3\text{-}R_4)(R_5)$ or C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkynyl, cycloalkyl, heteroalkyl, heteroaryl, $(CH_2)_n$-heterocycloalkyl, $(CH_2)_n$-aryl or $(CH_2)_n$-heteroaryl, wherein n is an integer from 1 to 4.

Substituents a, b, c and d can be either C or N, with the proviso that the total number of N atoms amongst a, b, c and d does not exceed 2.

[0038] Preferably, the above compounds are ones which bind specifically to a cell-bound or cell-free CB_2 receptor with an affinity characterized by a K_d of 100 μM or lower. Preferably, the compound is an agonist at the CB_2 receptor characterized by its ability to stimulate a CB_2-related post-binding signal transduction event after binding to a CB_2 receptor on a cell. The signal transduction event may be inhibition of adenylyl cyclase activity and/or induced cAMP generation.

[0039] Also provided are pharmaceutical compositions comprising (a) at least one compound as characterized above including a salt, derivative or prodrug and (b) a pharmaceutically acceptable carrier or excipient.

[0040] Another embodiment is directed to a method of treating an inflammatory condition, a cell proliferative disorder or an immune disorder, comprising administering to a subject in need of such treatment a therapeutically effective amount of a compound of any one of the above compounds or pharmaceutical compositions thereof.

[0041] Also included is a method of treating a subject having a disease or condition associated with abnormally low activity of, or signalling through, CB_2 receptors of cells of the immune system. Use of the compounds of Formula I and Formula II in this regard comprises administering to a subject in need of increased activity of or signaling through CB_2 receptors a therapeutically effective amount of at least one compound described above.

[0042] The above methods are used to treat one or more conditions or disorders selected from the group consisting of graft rejection, graft vs. host disease, T-cell mediated hypersensitivity including T cell-mediated dermatitis, allergic disease, arthritis, preferably rheumatoid arthritis, insulin-dependent diabetes mellitus (type I diabetes), multiple sclerosis, acute disseminated encephalomyelitis, asthma, chronic obstructive pulmonary disease, emphysema, bronchitis, acute respiratory distress syndrome, inflammatory bowel disease such as Crohn's disease, lupus or SLE, ischemic or reperfusion injury, celiac disease, atopic dermatitis, psoriasis, urticaria, scleroderma, mycosis fungoides, dermatomyositis, alopecia areata, chronic actinic dermatitis, stromal keratitis, eczema, Behcet's disease, Pustulosis palmoplanteris, Pyoderma gangrenum, Sezary's syndrome, systemic sclerosis, morphea, autoimmune thyroid disease, Addison's disease, an autoimmune polyglandular disease or syndrome, sialitis and Sjögren's syndrome, pernicious anemia, vitiligo, Guillain-Barre syndrome, glomerulonephritis and serum sickness.

[0043] In the foregoing methods, the compounds, preferably as pharmaceutical compositions, are administered orally, parenterally or topically. The compounds may be administered in combination with an agent that is also useful for the treatment of the symptoms or underlying cause of the disease or condition. Examples of such agents are methotrexate, sulfasalazine, a COX-2 inhibitor, hydroxy chloroquine, cyclosporine A, D-penicillamine, infliximab, etanercept , auranofin, aurothioglucose, sulfasalazine, sulfasalazine analogs,

mesalamine, corticosteroids, corticosteroid analogs, 6-mercaptopurine, cyclosporine A, methotrexate and infliximab, interferon β-1β, interferon β-1α, azathioprine, glatiramer acetate, a glucocorticoid and cyclophosphamide.

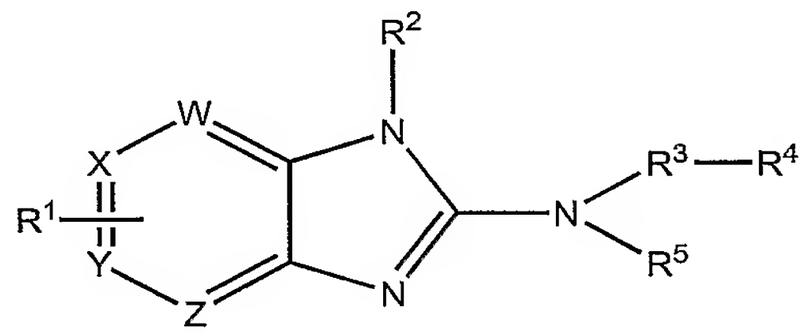
[0044] The invention provides a method for modulating (a) CB_2 receptors on a cell or (b) signal transduction mediated by CB_2 stimulation of a cell, comprising contacting the cell with a receptor-modulatory amount of at least one compound described above. In this method, the at least one compound is preferably one that activates CB_2 receptors. This method may also be performed using a compound that blocks the activation of said CB_2 receptors by an endogenous or exogenous CB_2 agonist.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0045] Disclosed herein are novel CB_2-R ligands that are potent inhibitors of autoimmune, inflammatory and nociceptive pathways and are therefore useful for preventing, treating or ameliorating diseases or conditions associated with undesired autoimmune reactivity. In particular, conditions or maladies mediated by T lymphocytes, inflammatory responses and pain may be overcome and attendant symptoms alleviated. These compounds and their uses are described and exemplified in detail below.

Chemical Structures

[0046] The central chemical entity upon which the novel compounds are based is shown in Formula I, below:



Formula I

In the preferred compounds of a preferred embodiment,

R^1 is: H, $C_{1\text{-}6}$ alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or $C_{2\text{-}6}$ alkoxy.

R^2 is: $C_{1\text{-}6}$ alkyl, cycloalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl, wherein n is an integer from 1 to 3.

R^3 is: CHR^6, CO or SO_2.

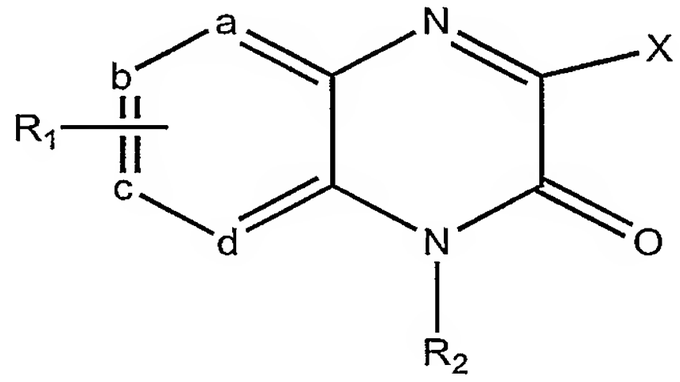
R^4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl.

R^5 is: H or lower alkyl or heteroalkyl.

R^6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy.

[0047] W, X, Y and Z can be either C or N, with the proviso that the total number of N atoms amongst W, X, Y and Z does not exceed 2.

[0048] In another preferred embodiment, the novel compounds are based on Formula II, below:



Formula II

In the preferred compounds of this embodiment,

R_1 is: H, C_{1-6} alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or C_{2-6} alkoxy.

R_2 is: C_{1-6} alkyl, cycloalkyl, $(CH_2)_n$-heteroalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl wherein n is an integer from 1 to 3.

X is: $N(R_3\text{-}R_4)(R_5)$, C(O)Y or C(NH)Y.

R_3 is: CHR_6, CO or SO_2.

R_4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl.

R_5 is: H or lower alkyl or heteroalkyl.

R_6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy.

Y is: $N(R_3\text{-}R_4)(R_5)$ or C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkynyl, cycloalkyl, heteroalkyl, heteroaryl, $(CH_2)_n$-heterocycloalkyl, $(CH_2)_n$-aryl or $(CH_2)_n$-heteroaryl, wherein n is an integer from 1 to 4.

Substituents a, b, c and d can be either C or N, with the proviso that the total number of N atoms amongst a, b, c and d does not exceed 2.

[0049] As used herein the term "alkyl" (as well as other groups having the prefix "alk", such as alkoxy, alkanoyl) denotes straight chain, branched, combinations of straight and branched, or cyclic fully saturated hydrocarbon residues. Unless the number of carbon atoms is specified, the term preferably

refers to C_{1-6} alkyl which is also referred to as "lower alkyl." When "alkyl" groups are used in a generic sense, *e.g.*, "propyl," "butyl," "pentyl" and "hexyl," *etc.*, it will be understood that each term may include all isomeric forms (straight, branched or cyclic) thereof. A preferred alkyl is C_{1-4} alkyl; more preferred is C_{1-3} alkyl. Examples of straight chain and branched C_{1-5} alkyl include methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, iso-pentyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, hexyl, heptyl, octyl, nonyl and the like.

[0050] "Cycloalkyl" means mono- or bicyclic or bridged saturated carbocyclic rings of 3 to 10 carbon atoms. The term also includes monocyclic rings fused to an aryl ring in which the point of attachment is on the non-aromatic portion. Examples of cycloalkyl include cyclopropyl or its substituted derivatives such as cyclopropylmethyl, cyclopropylethyl), cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, tetrahydronaphthyl, decahydronaphthyl, indanyl, and the like.

[0051] "Heteroalkyl" denotes a straight chain or branched chain saturated hydrocarbon residue that contains one or more heteroatoms including N, O or S, wherein the total number of atoms, inclusive of heteroatoms, does not exceed 20. Where two or more heteroatoms replace C atoms, these heteroatoms may be the same or different. The point of attachment of the heteroalkyl radical may be C, N, O or S. Thus, heteroalkyl encompasses primary, secondary and tertiary amine functionalities and ether and thioether functionalities. Examples of heteroalkyl include ethylamino, aminoethyl, propylamino, aminopropyl, isopropylamino, butylamino, aminobutyl, *tert*-butylamino, benzylamino, phenylethyamino, benzylmethylamino, benzylethylamino, dimethylamino, diethylamino, dipropylamino, dibutylamino, pyrrolidino, morpholino, piperazino, N-methylpiperazino, N-ethylpiperazino, dimethylaminoethylamino, diethyaminoethylamino, methoxy, ethoxy, propoxy, isopropoxy, butoxy, sec-butoxy, iso-butoxy, tert-butoxy and the like.

[0052] An alkyl group, as defined herein, may be optionally substituted by one or more substituents. Suitable substituents may include: halo (fluoro, chloro, bromo or iodo); haloalkyl (*e.g.,* trifluoromethyl, trichloromethyl); hydroxy; mercapto; phenyl; benzyl; amino; alkylamino; dialkylamino; cycloalkylamino;

arylamino; heteroarylamino; alkoxy (*e.g.*, methoxy, ethoxy, butoxy, propoxy phenoxy; benzyloxy, *etc.*); thio; alkylthio (*e.g.*, methyl thio, ethyl thio); acyl, for example acetyl; acyloxy, *e.g.*, acetoxy; carboxy (-CO_2H); carboxyalkyl; carboxyamide (*e.g.*, -CONH-alkyl, -CON(alkyl)$_2$, *etc.*); carboxyaryl and carboxyamidoaryl (*e.g.*, CONH-aryl, -CON(aryl)$_2$); cyano; or keto (where a CH_2 group is replaced by C=O).

[0053] The terms "alkoxy" and "acyloxy" refer to alkyl and acyl groups respectively when linked by oxygen.

[0054] As used herein the term "alkenyl" denotes groups formed from straight chain, branched or cyclic hydrocarbon residues containing at least one C=C double bond including ethylenically mono-, di- or poly-unsaturated alkyl or cycloalkyl groups as previously defined. Thus, cycloalkenyls are also intended. Unless the number of carbon atoms is specified, alkenyl preferably refers to C_{2-20} alkenyl. More preferred are lower alkenyls (C_{2-6}), preferably C_{2-5}, more preferably C_{2-4} or C_{2-3}. Examples of alkenyl and cycloalkenyl include vinyl, allyl, ethenyl, propenyl, isopropenyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl. Preferred alkenyls are straight chain or branched. As defined herein, an alkenyl group may optionally be substituted by the optional substituents described above for substituted alkyls.

[0055] As used herein the term "alkynyl" denotes groups formed from straight chain, branched, combinations of linear and branched, or cyclic hydrocarbon residues containing at least one C≡C triple bond including ethynically mono-, di- or poly- unsaturated alkyl or cycloalkyl groups as previously defined. Unless the number of carbon atoms is specified, the term refers to C_{2-20} alkynyl. More preferred are lower alkynyls (C_{2-6}), preferably C_{2-5}, more preferably C_{2-4} or C_{2-3} alkynyl. Examples include ethynyl, 1-propynyl, 2-propynyl, butynyl (including isomers), and pentynyl (including isomers), 3-methyl-1-pentynyl, 2-heptynyl, and the like. A particularly preferred alkynyl is a

C_{2-6} alkynyl. Preferred alkynyls are straight chain or branched alkynyls. As defined herein, an alkynyl may optionally be substituted by the optional substituents described above for alkyl.

[0056] The term "aryl" denotes a mono-, bi-, or poly-cyclic, conjugated or fused residue of an aromatic hydrocarbon ring system. The term also includes aryl group fused to a monocyclic cycloalkyl or monocyclic cycloheteroalkyl group in which the point of attachment is on the aromatic portion. Examples of aryl substituents are phenyl, biphenyl, naphthyl indanyl, indenyl, tetrahydronaphthyl, 2,3-dihydrobenzofuranyl, dihydrobenzopyranyl, 1,4-benzodioxanyl groups, and the like. An aryl group may be optionally substituted by one or more substituents as herein defined. Accordingly, "aryl" as used herein also refers to a substituted aryl.

[0057] The term "heteroaryl" denotes a mono-, bi-, or poly-cyclic conjugated or fused aromatic heterocyclic ring system, wherein one or more carbon atoms of a cyclic hydrocarbon residue is substituted with a heteroatom to provide a heterocyclic aromatic residue. Where two or more carbon atoms are replaced, the replacing atoms may be two or more of the same heteroatom or two different heteroatoms. Suitable heteroatoms include O, N, S and Se. Preferably, each ring of a bicyclic or polycyclic compound contains 5 or 6 atoms. Examples of heteroaryls include pyridyl, 4-phenylpyridyl, 3-phenylpyridyl, thienyl, furyl, furanyl, pyrrolyl, indolyl, imidazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl, thiazolyl, imidazolyl, triazolyl, tetrazolyl, triazinyl, thienyl, pyridazinyl, pyrazolyl, pyrazinyl, pyrimidinyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothiophenyl, benzothienyl, purinyl, quinazolinyl, phenazinyl, acridinyl, benoxazolyl, benzothiazolyl, benzimidazolyl and the like. As defined herein, a heteroaryl group may be optionally further substituted by one or more substituents as described above.

[0058] "Cycloheteroalkyl" means mono- or bicyclic or bridged saturated rings containing at least one heteroatom, preferably N, S or O, each of said rings having from 3 to 10 atoms in which the point of attachment may be carbon or nitrogen. The term also includes monocyclic heterocyclic rings fused to an aryl or heteroaryl group in which the point of attachment is on the non-aromatic portion. Examples of "cycloheteroalkyl" include pyrrolidinyl, piperidinyl, piperazinyl,

imidazolidinyl, 2,3-dihydrofuro(2,3-b)pyridyl, benzoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, dihydroindolyl, and the like. The term also includes partially unsaturated monocyclic rings that are not aromatic, such as 2- or 4-pyridones attached through the nitrogen or N-substituted-(1H,3H)-pyrimidine-2,4-diones (N-substituted uracils).

[0059] "Halogen" includes fluorine, chlorine, bromine and iodine.

[0060] When any variable radical (*e.g.*, R^1, R^2, *etc.*) occurs more than once in any constituent or in Formula I or II, its definition on each occurrence may be independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable, characterizable compounds.

[0061] The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally. "Independently substituted" means that the (two or more) substituents can be the same or different.

[0062] Compounds of Formula I or II may contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereoisomeric mixtures and individual diastereoisomers. Various embodiments of the disclosure encompass all such isomeric forms of the compounds of Formula I or II.

[0063] Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

[0064] Some of the compounds described herein may exist as tautomers, wherein the hydrogen atom is bonded at alternative sites. An example is a ketone and its enol form (keto-enol tautomers). Individual tautomers as well as mixture thereof are encompassed by the compounds of Formula I.

[0065] Compounds of the Formula I or II may be separated into diastereoisomeric pairs of enantiomers by, for example, fractional crystallization from a suitable solvent, for example methanol (MeOH) or ethyl acetate (EtOAc)or a mixture thereof. The pair of enantiomers thus obtained may be separated into

individual stereoisomers by conventional means, for example by the use of an optically active amine or acid as a resolving agent or on a chiral high performance liquid chromatography (HPLC) column. Alternatively, any enantiomer of a compound of the general Formula I or II may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

[0066] It is generally preferable to administer compounds of the present invention as enantiomerically pure formulations. Racemic mixtures can be separated into their individual enantiomers by any of a number of conventional methods. These include chiral chromatography, derivatization with a chiral auxillary functional group followed by separation by chromatography or crystallization, and fractional crystallization of diastereoisomeric salts. *See, for example*, B. M. Trost, *Stereocontrolled Organic Synthesis*, Blackwell Science,1994 (incorporated by reference in its entirety).

[0067] The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganese, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts.

[0068] Salts derived from organic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, trimethamine, and the like.

[0069] The term "pharmaceutically acceptable salt" further includes all acceptable salts such as acetate, lactobionate, benzenesulfonate, laurate, benzoate, malate, bicarbonate, maleate, bisulfate, mandelate, bitartrate, mesylate, borate,

methylbromide, bromide, methylnitrate, calcium edetate, methylsulfate, camsylate, mucate, carbonate, napsylate, chloride, nitrate, clavulanate, N-methylglucamine, citrate, ammonium salt, dihydrochloride, oleate, edetate, oxalate, edisylate, pamoate (embonate), estolate, palmitate, esylate, pantothenate, fumarate, phosphate/diphosphate, gluceptate, polygalacturonate, gluconate, salicylate, glutamate, stearate, glycollylarsenilate, sulfate, hexylresorcinate, subacetate, hydrabamine, succinate, hydrobromide, tannate, hydrochloride, tartrate, hydroxynaphthoate, teoclate, iodide, tosylate, isothionate, triethiodide, lactate, panoate, valerate, and the like. These can be used as a dosage form for modifying the solubility or hydrolysis characteristics of the inventive compounds, or can be used in sustained release, delayed release, or pro-drug formulations.

[0070] It will be understood that reference to a compound of Formula I or II is intended to include any pharmaceutically acceptable salt of the compound.

[0071] A compound embodying an aspect of this invention is a ligand and modulator of the CB_2-R. A given compound may be tested for its ability to bind directly to CB_2-Rs, to inhibit binding of a known ligand, and/or to determine the parameters of its binding (Bmax, Kd, etc.) using any conventional method. The compound may also be tested in an *in vivo* or *in vitro* model system, alone or in the presence of selective cannabinoid receptor antagonists, for (a) cannabimimetic activity and/or other biological outcomes related to the immune suppressive, antiinflammatory or antinociceptive objectives of the present invention.

[0072] The compounds of the preferred embodiments may be labeled with a detectable label. Many detectable labels are well known for use herein. General classes of labels which can be used in evaluating useful compounds include radioactive isotopes, paramagnetic isotopes, and compounds which can be imaged by positron emission tomography (PET), fluorescent or colored compounds, *etc.* Suitable detectable labels include radioactive, fluorescent, fluorogenic, or chromogenic labels.

[0073] Useful radiolabels (radionuclides), which are detected simply by gamma counter, scintillation counter or autoradiography include ^{3}H, ^{14}C, ^{35}S, ^{51}Cr, ^{125}I and ^{131}I. Other useful radionuclides are ^{99}Tc, ^{111}In, ^{97}Ru, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, ^{90}Y and ^{201}Tl.

[0074] Common fluorescent labels include fluorescein, rhodamine, dansyl, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine. The fluorophore, such as the dansyl group, must be excited by light of a particular wavelength to fluoresce. *See, for example*, R.P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, Sixth Ed., Molecular Probes, Eugene, OR, 1996).

[0075] *In situ* detection of the detectable label may be accomplished by removing a histological specimen from a subject and examining it by microscopy under appropriate conditions to detect the label. Those of ordinary skill will readily appreciate that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Bioassays for Cannabinoid Binding or Biochemical Activity

A. In Vivo

[0076] Numerous pharmacological effects are mediated via the cannabinoid receptors. Several basic notions embraced by the preferred embodiments are (1) pharmacological measures in animals that are representative of cannabinoid effects in humans (2) characterization of effects that are clearly attributable to cannabinoid ligands (*i.e.*, mediated through cannabinoid receptors), and (3) the practical consideration of selecting pharmacological effects that can be quantified and readily obtained. Using these criteria, pharmacological effects *in vivo* can be attributed to the activation of cannabinoid receptors as is discussed throughout this document.

[0077] *In vivo* systems for testing immune reactivity and its inhibition have been described in the Background section or are disclosed further below.

[0078] *Assay of Antinociceptive Activity*

Anti-nociceptive effects of the compounds may be tested using any known animal test for opioid mediated analgesia. Most commonly used in small animals, such as rodents, are the formalin test (inescapable painful stimulus), the measurement of tail flick latency (escapable painful stimulus) or paw lick latency in response to heat ("hot plate test") or a response to paw pressure. Chapman CR *et al.*, "Pain measurement: an overview." *Pain 22*:1-31, 1985, reviews and examines the practice and theoretical basis of pain measurement in animal

research, human subjects, laboratory investigation and clinical study. *See also*, Zimmermann M: "Ethical guidelines for investigations of experimental pain in conscious animals." *Pain 16*: 109-110, 1983; Coderre TJ, *et al.*, *Pain 18*:13-23, 1984; Rosland JH *et al.*, *Pharmacol Toxicol. 61*:111-1115, 1987. Larger animal tests are also known in the art. A laser-based method to measure thermal nociception in dairy cows is based on measuring behavioral responses (tail flicking, kicking) to a computer-controlled CO_2 laser beam applied to the skin on the caudal aspect of the metatarsus (Herskin MS *et al.*, *J. Anim. Sci. 81*:945-954, 2003).

[0079] *Formalin Test:*

Subcutaneous injection 25-100 µl of diluted formalin (in the range of 2.5-10% v/v in saline) is now a widely used rodent model for studying nociception. The injection generates behavioral responses that last from several minutes to up to more than 1 hour. Injection of formalin into the hindpaw of a rat induces a biphasic response in pain-related behaviors, such as C-fiber activation during phase 1, which triggers a state of central sensitization characterized by a long lasting phase 2. Pain related behaviors may be quantified by counting the incidence of spontaneous flinching of the injected paw or the duration spent in licking of the injected paw. Flinching is one pain-related behavior of the formalin model characterized by spontaneous, rapid, brief shaking or lifting of the paw. Accordingly, each episode of shaking, vibrating or lifting of the paw may be counted as one flinch. Flinching or licking may be used as a measure of assessing pain because either of them is more spontaneous than other formalin pain-related behaviors (*e.g.*, favoring) and consequently, is thought to be more reliable for this purpose. A nociceptive score is determined for each time block, *e.g.*, 5 minutes, by measuring the sum of duration of the behavior. A unit of one flinch may be defined by a single episode of lifting of the paw. Flinches may be counted for a total of 60 minutes at 5 minute intervals after plantar subcutaneous injection. *See, for example*, Dubuisson D *et al.*, Pain 4:161-174, 1977; Abbott FV *et al.*, Pharmacol Biochem Behav 15:637-640, 1981; Fanselow MS, Behav Neurosci 98: 79-95, 1984; Hara *et al.*, *Naunyn Schmiedebergs Arch Pharmacol. 326*:248-253, 1984; Shibata M *et al.*,: *Pain 38*:347-352, 1989; Wheeler-Aceto H *et al.*,

Psychopharmacology 104:35-44, 1991; Rosland JH, Pain 45:211-216, 1991; Coderre TJ *et al.*, *Pain 54*:43-50, 1993; Clavelou P *et al.*, *Pain 62*:295-301, 1995; Aloisi AM *et al.*, *Physiol Behav 58*:603-10, 1995; Lee, I-O *et al. Acta Anaesthesiol Sin 38*:59-64, 2000.

B. IN VITRO

1. Binding Assays

[0080] The most widely used radiolabeled cannabinoid receptor probe is [^{3}H]CP55940. Because CP55940 has approximately equal affinity for CB_1 and CB_2 binding sites (see Table 1), displacement assays with [^{3}H]CP55940 that are directed at characterizing the binding properties of novel unlabeled ligands are generally performed with membranes that are known to contain either CB_1 or CB_2-Rs but not both receptor types. These membranes are often obtained from cells transfected with CB_1 or CB_2-Rs. An alternative practice has been to use tissues that express dense populations of the receptor type naturally, usually spleen or other lymphatic tissue for CB_2-Rs. Although most cannabinoid receptors in the spleen are CB_2-Rs, some CB_1-Rs are expressed here as well (see below). A preferred specific assay is discussed in more detail below.

[0081] Cannabinoid receptor material and ligands are commercially available, for example, from Perkin-Elmer at the World Wide Web address: las.perkinelmer.com/. Cannabinoid Receptor Subtype CB_2 (human recombinant, HEK293 EBNA) is available for 100 assays under the Catalog # RBXCB_2M100UA or 400 assays (Catalog #RBXCB_2M400UA). The company's "Membrane Target Systems" are quality assured frozen membranes from cells that express recombinant or endogenous receptors and are tested in saturation radioligand binding to determine receptor density (B_{max}) and affinity (K_d) and pharmacological analyses. Membranes are carefully prepared and ready for a variety of HTS applications, including radioligand binding (using either proximity methods, such as FlashPlate®, or classical filtration methods) and fluorescence polarization methods. This vendor also provides [Side Chain-2,3,4-^{3}H(N)]-CP 55940 (1mCi (37MBq) under the Catalog # NET1051001MC. The molecular formula of this compound is $C_{24}H_{40}O_3$ and its molecular weight is 376 Da. The company materials reference the following publications: A.C. Howlett *et al.*, *Mol.*

*Pharmacol., 33:*297-302 (1988); W.A. Devane *et al.*, *Mol. Pharmacol., 34*:605 (1988); M. Herkenham *et al.*, *Proc. Natl. Acad. Sci, USA, 87:*1932 (1990).

[0082] Other commercially available probes with high affinity for CB_2-R include [^{3}H]HU-243, which binds equally well to CB_1 and CB_2-R (Devane *et al.*, 1992a; Bayewitch *et al.*, 1995), and [Side Chain 5,7-naphthyl 3H]-WIN55212-2, which has marginally greater affinity for CB_2 (Howlett et al., supra; Pertwee, 1999, supra). This material is also supplied by Perkin-Elmer under the Catalog #NET1058001MC. The molecular formula of this compound is $C_{27}H_{26}N_2O_3$ and its molecular weight is 426 Da.

[0083] Other radiolabeled ligands have been developed as potential probes for human single photon emission computed tomography (SPECT) or positron emission tomography (PET) experiments. These are ^{123}I-labeled analogs of AM251 and AM281 (Lan *et al.*, 1996; Gatley *et al.*, *Life Sci* **61:**PL191–PL197, 1997; Gatley *et al.*, *J Neurochem* **70:**417–423, 1998) and an ^{18}F-labeled analog of SR141716A (SR144385) (Barth, *Exp Opin Ther Patents* **8:**301–313).

2. Inhibition of cAMP Production

[0084] The ability of CB_2-R agonists to inhibit basal or drug-induced cAMP production is widely exploited for the quantitative, functional bioassay of cannabinoids *in vitro* (see, for example, Pertwee, *supra*). It is possible to achieve CB_2-selectivity by performing the assay in the presence of an adequate concentration of a selective CB_1 antagonist or by performing assays using cells transfected with CB_2-Rs (or membranes thereof). Cultured cells transfected with CB_2-Rs are particularly sensitive to the inhibitory effect of cannabinoids on cAMP production. Cells that express CB_2-Rs naturally (*e.g.*, mouse spleen cells and human blood lymphocytes) are relatively less sensitive to cannabinoid-induced inhibition of cAMP production (Pertwee, *Pharmacol Ther* **74:**129–180, 1997).

3. [^{35}S]Guanosine-5'-O-(3-thiotriphosphate) Binding Assay

[0085] This bioassay exploits the coupling of CB_2-Rs to G proteins. It relies on the increase in G protein affinity for GTP (and hence [^{35}S]GTPγS) that is triggered by the occupation by agonist molecules of CB_2-Rs, the measured response being net agonist-stimulated [^{35}S]GTPγS binding to G protein. The assay can be performed with the same range of tissue preparations that are used for the cAMP assay, again in the presence or absence of selective CB_1 antagonists. In addition, [^{35}S]GTPγS may be used in autoradiography experiments with tissue sections. To minimize background [^{35}S]GTPγS binding and maximize agonist-induced stimulation of binding, high amounts of GDP and sodium chloride are usually added to the bioassay system (*e.g.*, Breivogel *et al.*, *J Biol Chem* **273:**16865– 16873, 1998). Since GDP decreases basal binding of [^{35}S]GTPγS to a greater extent than agonist-stimulated binding, the overall consequence of adding GDP is an increase in net agonist-stimulated [^{35}S]GTPγS binding. The extent to which net agonist-stimulated [^{35}S]GTPγS binding can be enhanced in this way is limited by the concentration-related inhibitory effect that GDP has on absolute levels of both basal and agonist-stimulated binding. The [^{35}S]GTPγS assay is less sensitive than the cAMP and isolated tissue assays described herein. Presumably, this is because the measured responses in these other bioassays are located further along the signaling cascade than G protein, so that there is greater signal amplification. The [^{35}S]GTPγS assay should be independent of any

variations that may exist between tissues in the relative contribution made by different G protein-coupled effector mechanisms because it provides a total measure of G protein-mediated cannabinoid receptor activation rather than a measure of the activation of just one particular cannabinoid receptor effector mechanism as in the cAMP assay.

4. Various Practical Considerations in Assays

[0086] Cannabinoids are generally highly lipophilic and demonstrate relatively low water solubility. This low water solubility has prompted the development of the water-soluble cannabinoid receptor agonist O-1057 (Pertwee et al., Br J Pharmacol 129:1577–1584, 2000). The lipophilic nature of most CB_2-R binding agents, however, necessitates the use of nonaqueous vehicles for assessing their various activities. Commonly used vehicles for the *in vivo* or *in vitro* administration of cannabinoid receptor agonists and antagonists include ethanol, dimethyl sulfoxide, polyvinylpyrrolidone, Tween-80, 200 or 400, Cremophor, Emulphor, and bovine serum albumin (BSA). These are used singly or in combination, either by themselves or mixed with water or saline. Vehicle control experiments are important when the vehicle is suspected of having its own biological activity.

Measurement of Cellular Signal Transduction

[0087] Agonist stimulation of CB_2-R activates a number of signal transduction pathways via the Gi/o family of G proteins. In CHO cells expressing recombinant hCB_2-Rs, [^{35}S]GTPγS binding is stimulated by anandamide as a partial agonist compared with HU-210, whereas 2-arachidonoylglycerol is a full agonist. Free Giα proteins regulate adenylyl cyclase, leading to an inhibition of cAMP production. The consequent damping of phosphorylation by protein kinase A can modulate signaling pathways, such as that of ion channels and focal adhesion kinase. Values of potency and relative intrinsic activity may differ for the various signal transduction pathways.

1. Regulation of Adenylyl Cyclase

[0088] Inhibition of adenylyl cyclase has been characterized in human lymphocytes and mouse spleen cells expressing CB_2-R (see Howlett *et al.*, *supra*; Pertwee, 1997, 1999, *supra*, for review). The fact that cultured cell lines that express recombinant CB_2-Rs lead to inhibition of cAMP production indicates that

these receptor types are responsible for initiating this response. CB_2-R-mediateed inhibition of adenylyl cyclase is pertussis toxin-sensitive, further indicating the requirement for Gi/o proteins. The isoform of adenylyl cyclase expressed in cells may be a determinant of the outcome of cannabinoid receptor activation.

2. Regulation of Mitogen-Activated Protein Kinase (MAPK)

[0089] MAPK is activated in cultured human promyelocytic HL-60 cells possessing endogenous CB_2-Rs and in CHO cells expressing recombinant CB_2-Rs.

3. Signal Transduction via Ceramide

[0090] Primary astrocyte cultures showed that anandamide, Δ^9-THC, and HU-210 increased glucose metabolism, phospholipid synthesis, and glycogen synthesis via an SR141716A-inhibitable, but pertussis toxin-resistant mechanism. This type of response is also initiated by chronic stimulation of CB_2-Rs on cells of a susceptible C6 glioma strain and involves increased ceramide synthesis via serine palmitoyltransferase, Raf-1 activation, and MAPK (p42/44) activation.

4. Immediate Early Gene Expression and Protein Synthesis Regulation

[0091] MAPK activation can be linked to expression of immediate early genes such as Krox-24 (the expression of which is stimulated via CB_2-Rs in HL-60 promyelocytes (Bouaboula *et al.*, *supra*).

Screening of Cannabinoid Receptor Ligands for Binding

[0092] In a preferred embodiment, potential cannabinoid receptor ligands are screened for the ability to competitively inhibit the binding of a radioligand for the CB_2-R such as [3H]CP-55,940 or [3H]WIN-55,212-2 to membrane preparations isolated from HEK293 cells transfected with DNA encoding CB_2-R. In another embodiment, other types of cells derived from an animal, such as rat or mouse, or in continuous culture (or subcellular material) expressing the CB_2-R may be used. Such cells may naturally express the CB_2-R or may express it as a result of transfection with DNA encoding CB_2-R.

1. Membrane-Based Binding Assay

[0093] The following is a preferred assay using human CB_2-R and CB_1-R expressed in HEK293 cells. In round-bottom 96 well plates, test compounds are serially diluted (1:10; 100 µl/well) from 20 µM in assay buffer (50 mM Tris-HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 1 mg/ml BSA; pH 7.5) from stocks (2 mM)

prepared in propylene glycol. Five concentrations of each test compound are assayed in a final concentration range of 10^{-5} M to 10^{-9} M. Positive and negative controls (100 µl each, in triplicate) containing no inhibitor are included in each assay plate. To all wells, containing the test compound or the positive control, [^{3}H]WIN-55,212-2 (Perkin-Elmer Catalog #NET1058; specific activity= 41 Ci/mmol; 24.39 µM) is added, diluted in assay buffer to a concentration of 0.375 nM (80 µl/well – final concentration of 0.15 nM). To the negative control wells is added assay buffer (80 µl). To all wells is added the CB_2 membrane preparation (Perkin-Elmer Catalog #RBXCB2M; 20 µl/well), which has been previously diluted with assay buffer according to the manufacturers instructions. The plates are incubated for 1.5 hours at 37°C, before the membranes are harvested onto glass-fiber mats that have been pre-wetted (3x) with 0.05% poly(ethylene)imine (PEI). The mats are washed (10x) with distilled water and completely dried prior to adding scintillation cocktail (Microscint-O; Perkin-Elmer Catalog #6013611). Bound radioactivity of the plate(s) is measured in a Packard TopCount NXT microplate scintillation counter and the cannabinoid inhibitory activity of the test compound(s) determined (where activity is inversely proportional to the amount of radio-ligand bound). Non-linear regression analysis of the resulting data is performed using GraphPad Prism 4 (GraphPad, San Diego, Calif.).

[0094] This assay is advantageous as it can be conducted in a 96-well format that is readily automated. Different labeled cannabinoid ligands can be substituted into the assay. The recombinant cannabinoid receptors may be obtained from commercial sources and can be expressed in cultured CHO cells, HEK293 cells or insect (*e.g.*, *Spodoptera frugiperda*) cells.

2. Whole Cell Assay

[0095] Test compounds are serially diluted in assay buffer (50 mM Tris, 1 mM EDTA·4Na, 4mM $NaHCO_3$, , 0.2% BSA, 0.95% Hank's balanced salt solution (HBSS) at pH 7.4) from stocks prepared in propylene glycol. Aliquots (10 µl) are transferred into 96-well microtiter plates. Rodent spleen cells are collected, dispersed through a stainless steel mesh. Contaminating red blood cells in the preparation are then lysed (154 mM NH_4Cl, 12 mM $NaHCO_3$). The spleen cells are washed with "cell buffer" (2 mM EDTA·4Na, 3 mM $MgCl_2$, and 50 mM

Tris at pH 7.4) and resuspended in the same buffer at 2 x 10^7 cells/ml. Aliquots (50 µl) of cell suspension were added to each well of the microtiter plate.

[0096] The binding reactions are initiated by addition of WIN-55,212-2 (specific activity= 40-60 Ci/mmol) in assay buffer to each well of the microtiter plate (40 µl). Each 100 µl reaction mixture contains about 2.4 nM of the radioligand. Following incubation for 3 hours at 4°C, the membranes are harvested onto glass-fiber mats, that had been pre-wetted (3x) with 0.05% poly(ethylene)imine (PEI). The mats are washed (10x) with distilled water and completely dried prior to adding scintillation cocktail (Microscint-O). Bound radioactivity of the plate(s) is measured in a Packard TopCount NXT microplate scintillation counter and the cannabinoid inhibitory activity of the test compound(s) is determined (where activity is inversely proportional to the amount of radioligand bound). Non-linear regression analysis of the resulting data is performed using GraphPad Prism 4 (GraphPad, San Diego, Calif.).

Antibodies to CB_2-R

[0097] Polyclonal and monoclonal antibodies specific for cannabinoid receptors may be used for isolation and assay of receptor material or cells bearing receptor, for enumeration and identification of cells bearing receptors, *etc.* Such antibodies are commercially available. For example:

[0098] *A.* *Reagents from United States Biological (Swampscott, MA)*

1. Rabbit anti-rat CB_2-R polyclonal antibody, unconjugated
2. Rabbit anti-human CB_2-R polyclonal antibody, unconjugated

These antibodies are suitable for use in, *inter alia*, Western Blot, ELISA, and immunohistochemistry of paraformaldehyde-fixed tissues. Recommended dilutions for Western blots are 1–10µg/ml using ECL; for immunohistochemistry: 2–10µg/ml on paraformaldehyde fixed tissue; for ELISA: 1:10,000-1:100,000 using about 50–100ng control peptide per well. Optimal dilutions are be determined by the user.

[0099] *B.* *Reagents from Affinity BioReagents Inc. (Golden, CO)*

1. Unconjugated rabbit anti-human CB_2-R polyclonal antibody (Cat# PA1-744)

2. Unconjugated rabbit anti-rat CB_2-R polyclonal antibody (Cat# PA1-746)

These antibodies are designed for use in, *inter alia*, immunocytochemistry, immunohistochemistry, Western Blot, etc. They are provided as affinity purified IgG in PBS containing 50% glycerol, 1 mg/ml BSA and 0.05% sodium azide.

[0100] PA1-744 detects CB_2-R from human tissues and has been successfully used in Western blot and immunocytochemistry procedures. By Western blot, this antibody detects an ~60 kDa protein representing CB_2-R from human spinal cord homogenate. The antigen used to make the PA1-744 antibody is a fusion protein containing the first 33 amino acid residues from human CB_2-R

[0101] PA1-746 detects CB_2-R from human and rat tissues as well as transfected rat CB_2-R. PA1-746 has been successfully used in immunocytochemistry procedures. Immunocytochemical staining of AtT20 cells transfected with the rat CB_2-R gene with PA1-746 yields a pattern consistent with plasma membrane staining. The antigen used to make PA1-746 is a fusion protein containing the first 32 amino acid residues from rat CB_2-R.

[0102] *C.* *Reagents from CHEMICON International, Inc. (Temecula, CA)*

1. Affinity-purified polyclonal rabbit anti- CB_2-R (N-terminus) antibody, Cat# AB5640P

   Immunogen: Human CB_2-R, N-terminal peptide

2. Affinity-purified polyclonal rabbit anti- CB_2-R (C-terminus) antibody, Cat# AB5642P

   Immunogen: Rat CB_2-R, C-terminal peptide

[0103] AB5640P recognizes human CB_2-R. The immunogen shows no significant sequence homology with CB_1-R. The immunogen peptide is unique to human.

[0104] AB5642P recognizes rat CB_2-R. The immunogen shows no significant sequence homology with CB_1-R.. The immunogen peptide is 88% conserved in mouse and 82% in human. Reactivity with other species has not been confirmed.

Cellular Assays

(1) Monocyte/Macrophage Cytokine Production

[0105] Isolated or enriched human monocytes, freshly prepared, or the human monocytic cell line THP-1, are incubated at 10^6 cells/ml in RPMI 1640 media containing 10%FBS with the test compound for 30 minutes and then stimulated by the addition of either LPS (LPS) or immune complexes (IC). Cells are incubated for 6 h at 37°C. at which time the cell supernatants are removed and assayed for cytokines (TNF, IL-1β, IL-6, IL-8) using commercially available ELISA kits. Cannabinoid agonists inhibit the production of inflammatory cytokines.

(2) T Cell Proliferation Assays

[0106] The ability of the cannabinoids to inhibit the proliferation of normal human peripheral blood T cells that have been stimulated to grow with anti-CD3 plus anti-CD28 antibodies is evaluated. A 96-well plate is coated with a monoclonal antibody to CD3 (such as G19-4), the antibody is allowed to bind, and then the plate is washed. The antibody bound to the plate serves to stimulate the cells. Normal human peripheral blood T cells are added to the wells along with test compound plus anti-CD28 antibody to provide co-stimulation. After a desired period of time (*e.g.*, 3 days), [^{3}H]-thymidine is added to the wells, and after further incubation to allow incorporation of the label into newly synthesized DNA, the cells are harvested and counted in a scintillation counter to measure cell proliferation.

(3) Degranulation of Mast Cells

[0107] Cells of the basophilic leukemia line RBL 2H3 (an accepted model of mast cells or basophils) are cultured overnight in complete MEM at a concentration of 10^6 cells/ml at 37°C in 100 µl medium. Test compounds in 50 µl isotonic buffer are added and incubated for 2 hours at 37°C. Cell degranulation is triggered by the addition of 25 µl DNP-BSA IgE complex (300 ng/ml DNP-BSA) and incubated an additional 30 minutes at 37°C. Fifty µl of the cell supernatant from each well is removed and placed in a second 96-well plate which contains 50 µl of substrate solution [90 ml NAGA (hex) buffer (70 ml 0.2M $NaPO_4$, 20 ml 0.4M Citric Acid Monohydrate pH 4.5), 135 ml dH_2O, 615 mg p-nitrophenyl N-

acetyl D-glucosamine. The reaction is stopped by the addition of 100 µl NAGA stop solution (0.2M Glycine, 0.2M NaCl, 0.2M NaOH) and the plate read at a wavelength of 405 nm on a microtiter plate reader. The compounds of the Examples herein show a desired activity in the assays described.

[0108] A compound embodying a preferred aspect of this invention is a ligand and modulator of the CB_2-R, and as such, is useful in the prevention and/or treatment of a disease or condition associated with inappropriate activity or inactivity of the CB_2-R and any downstream signaling pathway linked thereto. Such diseases or conditions may be associated with alterations in the behavior of the CB_2-R itself, such as its level of cell surface expression, or with alterations in its intracellular signalling.

[0109] As used herein, the term "inhibit" includes its general meaning, *i.e.,* stopping, preventing, restraining, minimizing or slowing, reversing the progression or severity of symptoms of, a disease or disorder.

[0110] The term "treating" is intended to include "prevention," "protection from," "suppression" or "therapy" of a disease or disorder. "Prevention" generally involves administration of the one or more compounds based on Formula I or Formula II, or a pharmaceutical composition thereof prior to the induction or appearance of the disease. Thus, for example, in the animal model experimental autoimmune encephalomyelitis (EAE), successful administration of the therapeutic composition prior to injection of the encephalitogen (*e.g.*, myelin basic protein (MBP) that induces the disease results in "prevention" of disease. "Suppression" generally involves administration of at least one compound after the inductive event, but prior to the clinical appearance of disease. Again, using the EAE example, successful administration of a protective composition after injection of the encephalitogen, but prior to the appearance of neurological symptoms, comprises "suppression" of the disease. "Therapy" generally involves administration of at least one compound after the appearance of disease. In the EAE example, successful administration of a composition after injection of the encephalitogen, and after clinical signs have developed, comprises "therapy" of the disease. It will be understood that in human medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or not ascertained until well

after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "treatment" as used herein is meant to include "prophylaxis." As such, the present methods include both therapeutic and/or prophylactic administration of the compounds of the invention to "treat" a disease or condition.

[0111] Compounds of the Formula I and Formula II may be used to treat humans or other mammalian subjects. The compounds of the preferred embodiments are considered to be particularly suitable for the treatment of human subjects. Non-human subjects may include primates, livestock animals (*e.g.*, sheep, cows, horses, goats, pigs) domestic companion animals (*e.g.*, cats, dogs) laboratory test animals (*e.g.*, mice, rats, guinea pigs, rabbits) or captive wild animals.

[0112] Accordingly, another aspect of the preferred embodiments is a method for the treatment of a disease or disorder or symptoms mediated by, or influenced by, binding of an endogenous or exogenous ligand to the CB_2-R and subsequent activation of downstream signaling pathways and cellular activities. The method comprises administering to a mammalian subject, preferably a human, an effective amount of a compound of Formula I or II.

[0113] Compounds of Formula I and Formula II may be used to alleviate such diseases, disorders, conditions or symptoms including, for example: rejection of an allo- or xenotransplant (such as organ transplant); protection from ischemic or reperfusion injury such as that incurred during organ transplantation, myocardial infarction, stroke or other cause; induction of immunological tolerance to a transplant; an arthritic condition such as rheumatoid arthritis (RA), psoriatic arthritis or osteoarthritis; multiple sclerosis (MS); a respiratory and pulmonary disease including but not limited to asthma, chronic obstructive pulmonary disease (COPD), emphysema, bronchitis, and acute respiratory distress syndrome (ARDS); inflammatory bowel disease, including ulcerative colitis and Crohn's disease; lupus (SLE); graft vs. host disease; allergic including respiratory diseases (such as asthma, hay fever, allergic rhinitis); T-cell mediated hypersensitivity, including contact hypersensitivity (such as contact dermatitis including that caused by plant products such as poison ivy); other delayed-type hypersensitivity

responses; gluten-sensitive enteropathy (Celiac disease); psoriasis; urticaria or skin allergies; scleroderma; mycosis fungoides; dermatomyositis; alopecia areata; chronic actinic dermatitis; eczema; Behcet's disease; Pustulosis palmoplanteris; Pyoderma gangrenum; Sezary's syndrome; atopic dermatitis; systemic sclerosis; morphea; T cell- or antibody-mediated autoimmune diseases, including some of those mentioned above as well as autoimmune endocrine organ disease such as Hashimoto's thyroiditis, autoimmune hyperthyroidism such as Graves' disease, Addison's disease (autoimmune disease of the adrenal glands), an autoimmune polyglandular disease or syndrome, autoimmune hypopituitarism; Sjögren's syndrome; pernicious anemia; vitiligo; Guillain-Barre syndrome; glomerulonephritis; and serum sickness.

[0114] The phrases "administration of a compound" and/or "administering a compound" mean providing a compound of the invention or a prodrug thereof to a subject, preferably a human, in need of such treatment, by any acceptable route and in any effective dose. Combinations of compounds are also contemplated.

[0115] The therapeutic methods of the preferred embodiments include administration of an effective amount of at least one compound of Formula I or II to a subject in need of treatment or of prophylaxis of a disease or condition as disclosed herein. The need for a prophylactic administration is determined on the basis of well known risk factors. The effective amount of a compound is determined, in the final analysis, by a health care provider treating the subjection, and, as is well-known in the art, depends on factors such as the age, gender and weight of the subject, the precise disease to be treated, the severity of the disease, the presence of other diseases or conditions, the chosen route of administration, other concurrent drugs or treatments, and other factors according to the provider's judgment.

[0116] The utility of a compound of this invention may be demonstrated in an animal disease model that is known to be related to the disease or condition it is modeling. Examples of such animal disease models are: (1) experimental autoimmune encephalomyelitis (EAE), an inflammatory disease of the CNS that bears similarities to MS (*see, for example* Steinman, L., *Scientific American*, *269*:106-114 (1993).); (2) adjuvant arthritis (*see, for example*, Kayashima, K *et al.*, 1978, *J. Immunol. 120*:1127-1131), (3) streptococcal cell wall arthritis,

Mycoplasma arthritides arthritis and collagen-induced arthritis (see, for example, Pearson, CM, *Proc. Soc. Exp. Biol. Med. 91*:95 (1956); Cromartie, W.J. *et al., J. Exp. Med. 146*:1585 (1977); Trentham, D.E. *et al., J. Exp. Med. 146*:857 (1977); Chang, Y.H. *et al., Arthritis Rheum. 23*:62 (1980)).

[0117] The magnitude of a prophylactic or therapeutic dose or dose regimen of a given compound of Formula I or II will, of course, vary with the nature of the severity of the condition being treated and with the particular compound of Formula I or II and its route of administration. It will also vary according to the age, weight and response of the individual patient. A preferred daily dose range lies between about 1μg and about 100 mg per kg body weight, preferably between about 0.01 mg/kg to about 50 mg/kg, and most preferably between about 0.1 mg/kg to about 10 mg per kg, in single or divided doses. It may be necessary to use dosages outside these limits in some cases, as can readily be determined by one skilled in the art.

[0118] For intravenous administration, a suitable dosage range is between about 1 μg/kg/day and about 25 mg/kg/day, preferably between about 0.01 mg/kg/day and about 1 mg/kg/day.

[0119] Examples of effective amounts or doses given by oral administration are between about 0.01 mg/kg/day and about 100 mg/kg/day, preferably between about 0.1 mg/kg/day and about 10 mg/kg/day. The composition is preferably provided in the form of tablets containing from 0.01 mg to 1 g, preferably 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 15, 20, 25, 30, 40, 50 or 1000 mg of the active ingredient(s).

[0120] For the treatment of diseases of the eye, ophthalmic preparations for ocular administration preferably comprise from about 0.001% to about 1% by weight solution or suspensions of the compounds of Formula I or II in an acceptable ophthalmic formulation.

[0121] Also provided herein is a pharmaceutical composition which comprises at least one compound of Formula I or II and a pharmaceutically acceptable carrier. The term "composition," as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), preferably present in pharmaceutically effective amounts, and the inert ingredient(s), *i.e.,* pharmaceutically acceptable excipients, that make up the carrier, as well as any

product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, a pharmaceutical composition of the present invention encompasses any composition made by admixing at least one compound of Formula I or Formula II, one or more additional active ingredients, and pharmaceutically acceptable excipients.

[0122] The phrase "pharmaceutically effective amount" or a "therapeutically effective amount" of an active ingredient such as a compound of structural Formula I or II is intended to encompass amounts of the ingredient that will elicit the biological or medical response in a tissue, system, or animal (preferably human) that is being sought by the researcher or health care provider and are therapeutically or prophylactically useful in treating or preventing disease as described herein.

[0123] Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, suppositories and the like.

[0124] Pharmaceutical compositions embodying aspects of the invention may comprise a compound of Formula I or II as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier, and optionally, other therapeutic ingredients. By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient. In particular, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

[0125] Any suitable route of administration may be employed for providing a mammal, preferably a human, with an effective amount of a compound embodying an aspect of the invention. Oral and parenteral routes are included, examples of the latter being subcutaneous, intramuscular, intravenous, rectal, topical, buccal, ocular, intrapulmonary, intranasal, and the like. When given intravenously, the pharmaceutical composition may be injected or infused slowly, for example, by gravity infusion.

[0126] The compositions are formulated in a manner appropriate for each of said routes, including an ophthalmic preparation for ocular administration, an aerosol for inhalation for intrapulmonary or for nasal administration. The most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the pharmacy arts. See, for example *Remington's Pharmaceutical Sciences*, Gennaro, A.R., ed. 20th edition, 2000, Williams & Wilkins PA, (or latest edition).

[0127] For administration by inhalation, a compound of the present invention is conveniently delivered in the form of an aerosol spray from a pressurized pack or nebulizer. The compound may also be delivered as a powder which may be formulated; the powder composition may be inhaled with the aid of an insufflation powder inhaler device. Preferred delivery systems for inhalation are (1) a metered dose inhalation (MDI) aerosol, which may be formulated as a suspension or solution of the compound in a suitable propellant, such as a fluorocarbon or hydrocarbon, and (2) a dry powder inhalation (DPI) aerosol, which may be formulated as a dry powder of the compound with or without additional excipients.

[0128] Suitable topical formulations of a compound of the present invention Formula I or II include transdermal devices or, for dermal delivery, aerosols, creams, ointments, lotions, dusting powders, and the like. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Topical preparations containing the active drug component can be admixed with a variety of carrier materials well known in the art such as, *e.g.*, alcohols, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like. Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0129] The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholine.

[0130] Compounds of the present invention may also be delivered using an antibody, preferably a monoclonal antibody (mAbs) as a carrier to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide phenol, polyhydroxyethylasparamidephenone or polyethylene oxide-polylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, poly-ϵ-caprolactone, polyhydroxybutyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

[0131] Compounds of the present invention may also be delivered as a rectal or vaginal suppository employing bases such as cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

[0132] In practical use, the compounds of Formula I or II can be combined as the active ingredient(s) in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed. Useful examples include, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like, in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions. Carriers, such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like, may be used in the case of oral solid preparations such as, for example, powders, capsules and tablets. Solid oral preparations are preferred over liquid preparations. Because of their ease of

administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

[0133] Additionally or alternatively, administration may be by osmotic minipump, or by any other controlled release method or formulation, all of which are well-known in the art (*see, for example,* European Patent publications EP 092918, EP 0166596; U.S. Patents No. 3,536,809; 3,598,123; 3,630,200; 3,845,770; 3,916,899; 4,008,719; 4,789,516; 4,806,621; 4,877,606; 4,906474; 4,925,677 and 4,942,035; Hsieh, DS *et al., J. Pharm. Sci. 72:* 17-22 (1983); Kaitsu, I. *et al., J. Contr. Release 6*: 249-263 (1987); Goedemoed, J.H. *et al., Makromol. Chem. Symp. 19*: 341-365 (1988); Yang, M.B. *et al., Canc. Res. 49*:5103-5107 (1989); Greig, N. *et al., J. Contr. Release 11*:61-78 (1990); Jeyanthi, R. *et al., J. Contr. Release 13*:91-98 (1990); Saltzman, WM *et al., Polymer Preprints* 31-1: 2456 (1990)). The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

[0134] Pharmaceutical compositions embodying aspects of the invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient(s), as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active ingredient(s) with a carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing at least one active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredient(s) in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened

with an inert liquid diluent. Desirably, each tablet, capsule or cachet contains from 0.01 to 500 mg, preferably 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 3.0, 5.0, 6.0, 10.0, 15.0, 25.0, 50.0, 75, 100, 125, 150, 175, 180, 200, 225, and 500 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the subject being treated.

[0135] The dose may be administered in a single daily dose or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, based on the properties of the individual compound selected for administration, the dose may be administered less frequently, *e.g.*, weekly, twice weekly, monthly, *etc.* The unit dosage will, of course, be correspondingly larger for the less frequent administration.

[0136] When administered via intranasal routes, transdermal routes, by rectal or vaginal suppositories, or through a continual intravenous solution, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

[0137] Compounds of Formula I or II may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which compounds of Formula I or II are useful. Such other drugs may be administered, by a route and in an amount commonly used therefor, contemporaneously or sequentially with at least one compound of Formula I or II. When a compound of Formula I or II is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of Formula I or II is preferred. Accordingly, the pharmaceutical compositions of the preferred embodiments may include one or more other active ingredients, in addition to at least one compound of Formula I or II. Depending upon the particular condition to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may be administered together with therapeutic inhibitors of Formula I or Formula II.

[0138] For example, a compound of Formula I or II may also be combined with:

(1) an anti-inflammatory agent or agents such as a corticosteroid, a blocker of tumor necrosis factor-α (TNFα) action, an IL-1 receptor antagonist, azathioprine, cyclophosphamide and sulfasalazine;

(2) other immunomodulatory and immunosuppressive agents such as cyclosporin, tacrolimus, rapamycin, mycophenolate, mofetil, and an interferon;

(3) neuroactive agents such as acetylcholinesterase inhibitors, MAO inhibitors, anti-convulsants, ion channel blockers, riluzole, neurotrophic factors and anti-Parkinson's disease agents;

(4) agents for treating cardiovascular disease such as beta blockers, ACE inhibitors, diuretics, nitrates, calcium channel blockers, and statins;

(5) agents for treating liver disease such as corticosteroids, cholestyramine, interferons and anti-viral agents;

(6) agents for treating blood disorders such as anti-leukemic agents and various growth factors; and/or

(7) agents for treating immunodeficiency disorders such as gamma globulins.

[0139] Those additional agents may be administered separately from the composition that comprises at least one compound of Formula I or II, as part of a multiple dosage regimen. Alternatively, those agents may be part of a single dosage form, mixed together with at least one compound of Formula I or Formula II in a single composition.

[0140] The weight ratio of a compound of Formula I or II to a second active ingredient may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used. Thus, for example, when a compound of the Formula I or II is combined with a β_3 adrenergic agonist, the weight ratio of the compound of the Formula I or II to the β_3 agonist will generally range from about 1000:1 to about 1:1000, preferably about 200:1 to about 1:200. Combinations of at least one compound of Formula I or II and other active ingredients will generally also be within the aforementioned range, but in each case, an effective dose of each active ingredient should be used.

[0141] Treatment methods embraced by the preferred embodiments of this invention are based on the inventors' conception of modulating the CB_2-R and

treating CB_2-R mediated diseases using compounds based on Formula I or Formula II. Such treatment methods may comprise administering to a patient in need of such treatment a non-toxic, therapeutically effective amount of a cannabinoid compound that selectively activates the CB_2-R in preference to the CB_1-R or other GPCRs.

Immunological Studies in Vivo

[0142] The compounds embodying aspects of the invention are evaluated for their immunosuppressive effect using any acceptable *in vivo* form or model of immune reactivity or immune-mediated (such as autoimmune) disease.

[0143] One approach, exemplified herein, is inducing a cell-mediated immune reaction of the type that was once classified as a "type IV hypersensitivity" reaction.

[0144] The methods described herein are recognized and well understood by those skilled in the art and the principles of this can be found in many immunology text books such as A.K. Abbas *et al.*, eds., *Cellular and Molecular Immunology* (4th Ed.), W.B. Saunders Co., Philadelphia, 2000, C.A. Janeway *et al.*, eds., *Immunobiology. The Immune System in Health and Disease*, 4th ed., Garland Publishing Co., New York, 1999; Roitt, I. *et al.*, eds, *Immunology*, 5th ed., C.V. Mosby Co., St. Louis, MO (2001); Klein, J. *et al.*, *Immunology*, 2nd edition, Blackwell Scientific Publications, Inc., Cambridge, MA, (1997).

[0145] An animal, preferably a rodent, may be sensitized to a self protein (in practice skin is the easiest tissue to use) by chemically modifying the protein(s) in this tissue thereby allowing it to be 'seen' as 'foreign' tissue by the immune system. This is done by exposing the skin to a reactive 'hapten' which is usually an alkylating or arylating agent that reacts covalently with and thereby modifies protein(s) in the skin. Alternatively, the animal may be immunized by a foreign protein, such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) or other conventional antigen, or by allogeneic or xenogeneic cells ("transplant").

[0146] Before, at the time of, or after sensitization or immunization, the compound being evaluated is administered to the animal by whichever route is desired at selected doses and intervals. Once sufficient time has lapsed for

immunity to develop (in a control animal), the animals are challenged to assess their state of immunity.

[0147] In the case of contact hypersensitivity, animals are challenged by painting the ear pinnae with areactive hapten. The delayed hypersensitivity (DH) response is measured by measuring ear thickness, which is a reflection of local recognition of antigen by antigen presenting cells, their activation and recruitment of T cells to the reaction site, and the release by the T cells or other effector cells of various mediators, typically cytokines, which cause an influx and extravasation of other inflammatory cells and fluids, resulting in thickening of the ear. Other forms of delayed hypersensitivity may be evoked and observed as skin tests where challenge antigen is given subcutaneously or intradermally, and the local DH response evaluated as erythema and induration of the area. This is preferred with protein antigens. In the case of a foreign tissue antigen, the animal may receive a skin graft or other tissue or cell graft as a challenge.

[0148] Antibody responses are induced using any standard immunization protocol with an immunogen of choice, and optionally, an adjuvant. Treatment with the test agent may be initiated at various times before, as well as, optionally at the time of and after, initial immunization. Animals may be rested and boosted with a second or additional administration of antigen. At appropriate times thereafter, with or without continuation of treatment with the test agent, the animals are bled, and serum samples are assayed for antibody levels using any conventional immunoassay, *e.g.*, and enzyme immunoassay (EIA) such as ELISA. Alternatively or additionally, blood cell or other lymphocytes may be harvested from the animal (either while keeping the animal alive or at termination) and tested for their secretion of antibodies using a plaque assay or ELISPOT assay, all of which are conventional in the art.

Inhibition of Cell-Mediated Immune Disease of the Central Nervous System

[0149] Experimental autoimmune (or allergic) encephalomyelitis (EAE) is an inflammatory disease of the central nervous system (CNS) that bears similarities to multiple sclerosis (MS) and has therefore been used extensively as an animal model of MS. Many references to this model are available in the medical and scientific literature. *See, for example*, Paterson, P.Y., In *Textbook of*

Immunopathology (Miescher, P.A. *et al.*, eds) Grune & Stratton, New York, 1976, especially pp. 179-213; Alvord, E.C. Jr., In: *Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis* (Alvord, E.C., ed.), Liss, New York, 1984, pp. 1-511; Steinman, L., *Scientific American*, 269:106-114 (1993). The pathology of EAE is characterized by an influx of lymphocytes and monocytes into the brain and spinal cord with an associated demyelination of the CNS neurons (Raine, C.S. *et al. Lab Invest. 43*:150-157 (1980); Paterson, P.Y *et al.*, *Immunol. Rev.* 55:89-120 (1980)) resulting in partial or complete paralysis, and in severe cases, death. Neural antigen-specific $CD4^+$ T lymphocytes are the initiators of the response because*in vivo* depletion of $CD4^+$ T cells inhibits induction of EAE (Waldor, M.K. *et al.*, *Science 227*:415-417 (1985). Only $CD4^+$ T cell lines or clones can passively transfer the disease (Holda, J.A. and Swanborg, R.H., *Europ. J. Immunol. 12*:453-455; Ben-Nun, A. and Cohen, I.R. *J. Immunol. 129*:303-308). Thus, the disease may be characterized as being T lymphocyte-mediated and tissue-specific.

[0150] Compounds embodying aspects of the invention which inhibit induction or pathogenesis of EAE are expected to be useful for treating MS or other cell-mediated diseases of the CNS, both in humans and in nonhuman mammals.

To study the effect of the present agents *in vivo* on a disease that includes in its pathogenesis the migration of T lymphocytes into a specific tissue (such as the brain or spinal cord), experiments are performed in the Lewis rat model of passively (adoptively) transferred EAE. Myelin basic protein (MBP)-specific T lymphocyte cell lines suitable for disease transfer are generated from the draining lymph nodes (LNs) of Lewis rats previously immunized with MBP in complete Freund's adjuvant (CFA) essentially according to the method of Ben-Nun *et al.*, 1981, *supra*. Primary lymphocyte isolates for passive transfer of EAE are generated from naïve donor rats according to the method of Panitch and McFarlin (Panitch, H.S. and McFarlin, D.E., *J. Immunol. 119*:1134-1137), incorporated herein by reference

[0151] Donor T lymphocytes are generated from female 10 to 12 week old Lewis rats weighing 150 to 200 g that have been injected intradermally in each hind footpad with a 100 µl volume CFA containing guinea pig myelin basic

protein (gpMBP) (the purified protein is prepared according to Deibler, G.E. *et al.*, *Prepar. Biochem. 2*:139-165 (1972), incorporated herein by reference). The adjuvant emulsion is prepared by emulsifying the oily component, (consisting of a mixture of light mineral oil (Sigma) containing 15% mannide monooleate (Sigma) and finely ground *Mycobacterium butyricum* (4 mg/ml; Difco)), with an equal volume of a solution of gpMBP (0.25 mg/ml) in normal saline. Thus, each rat receives a total dose of 25 μg of MBP and 400 μg of *M. butyricum.*

Approximately 11 days following injection the rats are euthanized and the draining lymph nodes (LNs) (popliteal and inguinal) are removed aseptically by blunt dissection and placed into lymphocyte culture medium for the preparation of encephalitogenic T cell lines. The spleens are removed aseptically and placed into lymphocyte culture medium for the preparation of primary isolate lymphocytes for passive transfer. This protocol was used as described to successfully isolate T lymphocytes.

For preparing cell lines, a single cell suspension is prepared from the LNs. The cells are washed in culture medium and any red blood cells are lysed in the usual fashion with ammonium chloride solution. These lymphocytes, at concentration of about 5 x 10^6 /ml are cultured for about 72 hours in the presence of MBP (*e.g.*, 0.06 mg/ml) at 37°C in a humidified atmosphere containing 7.5% carbon dioxide. The cells are collected and the lymphoblasts are isolated by centrifugation on a Ficoll® (Pharmacia, Uppsala, Sweden) gradient in an identical manner to that described by Ben Nun et al. supra. The fraction containing ~90% lymphoblasts are cultured further in complete DMEM to which is added (15% v/v) a culture supernatant containing a mixture of growth factors (e.g., crude supernatant of Con A-stimulated lymphocytes or purified T cell growth factors), 10% fetal calf serum, and nonessential amino acids. No antigen (MBP) is added. The cells are plated in, e.g., 100 mm petri dishes at a concentration of about 2 x 10^5 cells/ml and replated every 3 or 4 days. Prior to transfer into Lewis rat recipients, the cells are restimulated with MBP (0.01 mg/ml), and irradiated syngeneic antigen-presenting cells such as thymocytes, for about 4 days. These cultured, stimulated T lymphocytes are highly encephalitogenic: as few as 5x10^5 cells can induce disease in naïve Lewis rats.

[0152] Primary isolate cells for passive transfer of EAE are derived from the isolated spleens (above) by preparing single cell suspension of lymphocytes and placing these in lymphocyte culture medium with 10% FCS at a concentration of about $2x10^6$ cells/ml. Concanavalin A is added at a final concentration of, for example, 4 μg/ml. The cells are cultured at 37°C in an atmosphere of 5% CO_2 for about 72 hours. After harvesting the cultured lymphocytes (and washing twice in Hank's balanced salt solution (HBSS)), the cells are resuspended in HBSS and injected intravenously into the lateral tail vein of recipient rats, each of which receives a selected number of cells, for example, between 50 and 70 x 10^6 cells in a volume of 200 ml, by injection into the lateral tail vein. Drug treatment is initiated on the day following cell transfer. In a typical experiment, a group of 5 female Lewis rats, approximately 9 weeks old, weighing 110±15 grams receives encephalitogenic lymphocytes as described and treatment with the cannabinoid receptor ligand is instituted 24 hours later with twice daily intraperitoneal (i.p.) injections such that animals received the agent at a daily dose rate of 10 to 100 mg/kg. Typically a group of control rats of the same age, sex and weight receive twice daily ip injections of the drug diluent or vehicle beginning 24 hours after encephalitogenic cell transfer. The above protocols were followed as described to generate test animals for assessing the novel cannabinoids discussed herein.

[0153] Therapeutic efficacy of the cannabinoid ligand is determined by clinical assessment of disease severity. It is usual for the disease to present with an ascending paresis followed by paralysis beginning with the distal tail. The disease is often scored on the basis of severity of symptoms. In the present discovery the clinical disease was scored daily beginning on day 4 following cell transfer using the following numerical scoring system: 0 – no clinical signs; 1 – paresis with flaccidity of the distal half of the tail; 2 – flaccidity of entire tail; 3 – ataxia and difficulty with righting; 4 – frank hind-limb paresis; 5 – hind-limb paralysis. An additional measure of disease severity is based on histological examination of tissue sections taken from the spinal cord of EAE affected animals. This method is based on scoring the number and severity of inflammatory lesions in the tissue. It is known that in most cases the severity of disease symptoms is positively correlated with the number and severity of inflammatory lesions in the CNS of EAE-affected animals. In the present study, qualitative assessment of lesions

were assigned numerical values, and thus, a mild lesions were scored as 1, moderate lesions 2 and severe lesions 3. Histological sections were prepared from 14 mm sections of spinal cord (lumbar and sacral regions) taken from 10% neutral buffered formalin-perfused animals. The cords were embedded in paraffin, sectioned (5 μm) and stained with hematoxylin plus eosin.

Inhibition of Cell-Mediated Immune Disease of Synovial Tissue
(Passively-Transferred Adjuvant Arthritis)

[0154] Passively transferred adjuvant arthritis (AA) is a T lymphocyte-mediated disease in which T cells from an animal with active arthritis are transferred to a naïve syngeneic recipient to induce disease. The naïve recipient subsequently develops clinical signs of disease, including lymphocyte migration into the synovium with subsequent swelling of affected joints. The immunological nature of this disease and its dependence upon T lymphocytes has been well established for many years. (See, for example, Kayashima, K. *et al.*, 1978, *J. Immunol. 120*:1127-1131; Waksman, B.H. *et al.*, 1963, *Int'l. Arch. Allergy 23*:129-139; Pearson, *supra*;; Whitehouse, D.J. *et al.*, 1969, *Nature 224*:1322.)

[0155] This model is suitable for studying the effect of a compound of Formula I or Formula II *in vivo* in a model of rheumatoid inflammatory disease. Male DA (Dark Agouti) rats, 8 to 12 week old, are immunized with six 50 μl injections of a suspension of *Mycobacterium butyricum* in mineral oil intradermally at the base of the tail, plus four 50 μl injections at the base of the dorsal neck. The suspension is prepared by mixing, e.g., 6 mg/ml of *M. butyricum* (Difco Laboratories, USA) that has been ground to a fine powder using a mortar and pestle in light mineral oil (Sigma). About ten days after immunization, the rats are euthanized and their spleens removed aseptically. A single cell suspension of lymphocytes is prepared in the usual manner and placed in lymphocyte culture medium with 10% FCS at a concentration of about $2x10^6$ cells/ml. Con A is added at a final concentration of, for example, 4 μg/ml. The cells are cultured at 37°C in an atmosphere of 5% CO_2 for about 72 hours. After harvesting the cultured lymphocytes (and washing twice in HBSS), the cells are resuspended in HBSS and injected intravenously into the lateral tail vein of recipient rats, each of which receives a selected number of cells, for example,

between 5 and 10 x 10^7 cells, *e.g.*, 8.5 x 10^7 cells, in a volume of 0.5 ml. Drug treatment is initiated on the day of cell transfer. The test compound of is administered intraperitoneally at a dose of about 10-100 mg/kg/day.

[0156] After 5 to 8 days, a characteristic thickening and cutaneous hyperemia of the distal joints of the hind legs becomes clinically apparent in saline-treated control animals. Disease severity is evaluated and graded in each animal by daily measurement of the mediolateral widths of both ankle joints. The data are expressed as the mean of the change (compared with width prior to cell injection) in mediolateral ankle width expressed in millimeters (± standard error of the mean).

Other Models of Arthritis and Autoimmune Disease

[0157] The compounds of the present invention are administered to rodents in several well-known animal models of arthritis and autoimmune disease. These include adjuvant arthritis (see above), streptococcal cell wall arthritis, *Mycoplasma arthritides* arthritis and collagen-induced arthritis. (*See, for example*, Pearson, *supra*; Cromartie, W.J. *et al., J. Exp. Med. 146*:1585 (1977); Trentham, D.E. *et al., J. Exp. Med. 146*:857 (1977); Chang, Y.H. *et al., Arthritis Rheum. 23*:62 (1980)).

1. Streptococcal Cell Wall Arthritis Model (see: Schwab, JH *et al., J. Immunol. 150*:4151 (1993))

A. Induction, measurement, and treatment of arthritis

[0158] Female Lewis rats weighing about 175g are injected intraarticularly (i.a.) under ether anesthesia above the calcaneus through the Achilles tendon into the tibiotalar (ankle) joint on day 0 with 2.0 μg of rhamnose equivalents (approximately 6.0 μg dry weight) of peptidoglycan-polysaccharide from cell wall of group A *Streptococci* (PG-APS) suspended in 10 μl of pyrogen-free saline, as described previously (Esser, R.L., *et al., Arthritis Rheum. 28*:1402 (1985); Stimpson, S.A., *et al.*, In: *Pharmacological Methods in the Control of Inflammation*, J. Chang *et al.*, Eds. Alan R. Liss, Inc., New York, p. 381 (1989)). Right or left joints are injected with PG-APS in alternate animals, and contralateral joints are injected with 10 μl of pyrogen-free saline. The lateral diameter of the ankle joint is measured with a Fowler Ultra-Cal II digital caliper (Lux Scientific Instrument Corp., New York, NY). The average of three

measurements for each joint is recorded. Results are presented as the mean ± SE of the increase in joint diameter (difference between pre- and postreactivation).

[0159] B. Histopathology: Rats are sacrificed and the ankle joints are removed, skinned, fixed in formalin, decalcified, embedded in paraffin, sectioned sagitally, and stained with hematoxylin-eosin. The significance of differences between groups is assessed by Student's two tail *t*-test.

2. Actively-Induced Adjuvant Arthritis Model (See, Chang *et al., supra*)

[0160] Male Lewis rats weighing 235-250 gm are used. Freund's complete adjuvant is either purchased commercially or prepared by grinding powdered *Mycobacterium butyricum* (10 mg; Difco Laboratories) with mineral oil (1.01 ml; Primol 355, Hampden Color Chemical Company). Adjuvant arthritis is produced by a single intradermal injection of the adjuvant into the tail or one hindpaw. The dose is about 0.5 mg heat killed *Mycobacterium tuberculosis* (*Mt*) suspended in 100 µl IFA. The volume of the uninjected hindpaw is measured by the method of Winter *et al., Proc. Soc. Exp. Biol. Med. 111*:544 (1962) on day 0 and 16 (with respect to the injection of adjuvant). The increase in the volume of the uninjected hindpaw serves as a measure of arthritis.

[0161] To determine the effect of a therapeutic composition comprising at least one compound of Formula I or Formula II, rats are treated with either vehicle or the composition dissolved or suspended in vehicle at the required concentration each day from day -1 to day -15 (with respect to adjuvant injection). The initial paw volume (V_I) is measured on the day of adjuvant injection. Sixteen days later, the volume (V_F) of the uninjected hindpaw is measured. Percent inhibition is calculated according to the following equation:

$$\% \text{ inhibition} = 1 - \frac{V_F \text{ drug} - V_I \text{ drug}}{V_F \text{ control} - V_I \text{ control}} \times 100$$

[0162] Alternatively, severity of arthritis is assessed by scoring each paw from 0 to 4 based on degree of swelling, erythema, and deformity of the joints. Thus, the maximum possible arthritis score is 16.

3. Collagen Type II-Induced Arthritis (CIA) Model (see Trentham *et al., supra*)

[0163] Sensitization Procedures. Collagen is dissolved in 0.1M acetic acid at a concentration of 1mg/ml. Equal volumes of collagen solution and CFA or IFA are mixed and emulsified. One ml of the cold emulsion is immediately injected intradermally in four to six sites on the backs of the rats. Small ulcers frequently form at the injection site, but these heal without sequelae in 7-10 days. Control injections consist of (a) acetic acid emulsified in CFA (Complete Freunds Adjuvant) or IFA (Incomplete Freunds Adjuvant) or (b) human or chick type II collagen dissolved in acetic acid and injected intradermally without adjuvant. As an additional control, 1.0 ml of $MgCl_2$-extractable cartilage proteoglycans containing approximately 200 μg uronate per ml is mixed with 0.5 ml of CFA or IFA, emulsified, and injected as with collagens. Unless otherwise specified, booster doses consisting of 0.5 mg collagen dissolved in 0.5 ml 0.1 M acetic acid are given ip without adjuvant 21 days after primary immunization. One ml of the $MgCl_2$ extract is given ip after an identical interval to the proteoglycan control animals.

[0164] Arthritis Evaluation. Animals are observed daily for the onset of arthritis, and an arthritic index is derived by grading the severity of involvement of each paw from 0 to 4. Scoring is based on the degree of periarticular erythema and edema as well as deformity of the joints (Wood, F.D., *et al., Int. Arch. Allergy Appl. Immunol. 35*:456 (1969)). Swelling of hindpaws is also quantitated by measuring the thickness of the ankle from medial to lateral malleolus.

4. Autoimmune Model MRL/lpr Mice (see: Kim, C. *et al., J. Exp. Med. 174*:1431-1437 (1991))

[0165] MRL/Mp-lpr/lpr mice (4-6 wks old) may be purchased from the Jackson Laboratory (Bar Harbor, ME) or other supplier.

ELISA for Anti-DNA Antibodies and Immune Complexes

[0166] Polystyrene microtiter wells are coated with double-stranded DNA (ds-DNA) or goat C1q. Blood is obtained from individual mice before the biweekly injections. Sera are diluted in 0.05% Tween-20 in PBS at a 1:500 dilution and allowed to incubate in the plates for 60 min at room temperature. The plates are then washed with PBS-Tween, and 50μl of 1/1000 dilutions of goat anti-mouse IgG and IgM antibodies conjugated to urease (or another enzyme that is known to be useful in EIA) are added to the plates. After incubation for 30

min., the plates are washed three times with PBS-Tween and twice with 0.15M NaCl. The plates are then incubated with a solution of the chromogenic substrate for the enzyme. Colorimetric change is quantified by measuring absorbance at the appropriate wavelength for the particular colored product of the enzymatic reaction using a microplate reader.

Proteinuria and Physical Symptoms

[0167] Urine is obtained from mice. Protein concentration and the presence of blood in urine are measured semiquantitatively by commercial reagent strips for urinalysis.

[0168] Physical symptoms are visually scored as: 0, no symptoms; 0.5, trace; 1-4, when visible symptoms are observed, with 4 being the most severe (physical symptoms include lymphadenomegaly, immune complex vasculitis, and necrosis of the ears). Scores representing physical symptoms are calculated by determining the total score for each group and then dividing by the number of animals alive in that group when the measurement is taken.

Treatment

[0169] For each of the models described above, treatment with at least one compound based on Formula I or Formula II, or compositions thereof, is started 6-14 days after the injection of the inducing agents (or in the case of MRL/lpr mice beginning at 4 weeks of age). Doses vary from 1 μg to 100 mg of the test compounds.

[0170] The compounds are administered i.v. or i.p. at 1 week intervals for 4 weeks. Outcomes are assessed as described above. For all arthritis models outcome measures include: (a) quantitative measurement and grading of joint swelling erythema or deformity, and (b) assessment of histopathology of joints using a quantitative grading system.

RESULTS

[0171] In all the models described, evidence indicated that CB_2-R agonist compounds defined by Formula I and Formula II are effective in significantly reducing direct or indirect measures of arthritis.

Treatment of Rheumatoid Arthritis in Humans

(See: W.J. Koopman (ed) *Arthritis and Allied Conditions: A Textbook of Rheumatology*, Lippincott, Williams & Wilkins; 13th edition, 1996.)

Treatment Procedure

[0172] Doses of the compounds of Formula I or Formula II are determined as described above using, *inter alia*, appropriate animal models of autoimmune disease.

[0173] A treatment consists of injecting a patient with 0.1, 1, 10 or 100 mg of the compound iv or subcutaneously, or infusing the compound iv in 100 ml of normal saline over a 30 minute period daily for between three and six weeks. Treatment can also consist of oral dosing with 0.1, 1, 10, 100 or 500 mg of the compound once, twice or three times daily for three to six weeks. Clinical responses are assessed by the criteria described below. Treatments are continued in patients with stable or exacerbating disease. Treatment generally may be administered on an outpatient basis.

Clinical Outcome Measures

[0174] Outcome measures used to assess treatment efficacy in rheumatoid arthritis (RA) should detect the smallest *clinically important* change and, at the same time, be reliable and valid with respect to capturing the dimensionality of the clinical and pathophysiologic responses. To avoid bias, both patients and assessors preferably are blinded during testing.

[0175] The methods most commonly used are based on quantification of cardinal features: pain, swelling, heat and redness. Laboratory tests may also be used in assessment, though a treatment that only reduces a laboratory measure without, for example, relieving joint pain is of less interest. No single ideal method is known to accurately reflect disease activity in arthritis. As a result, it is useful to aggregate end points into a composite index. Composite indices are constructed by statistical or judgmental procedures that allow aggregation of scores assigned to different end points.

[0176] Objective and sensitive measurements are preferred to subjective ones. One sensitive parameter to change with antirheumatic drug therapy in RA is the patient's subject assessment of pain relief. Objective measurements include radionuclide joint uptake. Others are the 50-foot walking time and assessment of functional disability (the second most important symptom in arthritis). Examples of useful outcome measures appear in Table 3, below.

[0177] Because pain is the major complaint of the rheumatic sufferer, measurement of pain relief is important in assessing clinical response to the therapeutic composition or method of this invention. Adjectival scales may be used with numeric values given to the adjectival scale, for example: 0= no pain, 1= slight pain, 2= moderate pain, 3= severe pain, and 4= extremely severe or agonizing pain. Such a scale is known to discriminate between nonsteroidal anti-inflammatory analgesics and placebo in short-term trials (Lee, P., *J. Rheumatol.* *3*:283-294 (1976)). Other methods of measuring pain include assessment of pain threshold and pain tolerance (Huskisson, E.C., *Clin. Rheum. Dis.* *2*:37-49(1976)).

[0178] A simple count of clinically active joints, as determined by pain on passive motion, tenderness on pressure, or inflammatory joint swelling is used (Cooperat. Clin. Comm. Amer. Rheum. Assoc., Clin. Pharmacol. Ther. 8:11-38 (1967)) to score joint tenderness. Also, firm digital pressure is applied to the joint

margins and the degree of tenderness is graded by the patient's response. Lansbury's Articular Index (Lansbury, J., *Arthritis Rheum. 1*:505-522 (1958)) is useful in assessing progress. Scoring a few selected "signal" joints may permit better assessment of therapeutic effect than a total joint count. A standardized dolorimeter tested against the Lansbury indices is highly reproducible. The Ritchie Articular Index (RAI) is based on summation of joint responses after firm digital pressure. The responses are recorded as 0= no tenderness, +1= patient says it is tender, +2= patient says it is tender and winces, and +3= patient says it is tender, winces, and withdraws limb. The sum of this Index is 78 and reflects exacerbations of disease and improvement induced by antirheumatic drugs. This index correlates with the patient's assessment of pain, in the upper limbs with grip strength, and in the lower limbs with the time to walk 50 feet.

[0179] Various instruments are available to measure grip strength which is determined by the strength of the muscles in the forearm and hand, and the pain and degree of joint destruction in the wrist, hand, and finger joints; grip strength correlates with the RAI.

[0180] The range of motion of peripheral joints in normal subjects is known, and these measures have been assessed in studies of ankylosing spondylitis. Spinal movement is measured by several methods including the Dunham spondylometer (Anderson, J.A.D., *Clin. Rheum. Dis. 8*:631-653 (1982)), skin distraction (Moll, J.M.H. *et al., Rheum. Phys. Med. 11*:293-312 (1972)), an inclinometer (Domjan, L. *et al., Hung. Rheum. 28 (Suppl)*:71-76 (1987)). Timing of certain movements or set maneuvers related to activities of daily living, are useful, in particular the time to walk 50 feet (Lee, *supra*; Grace, E.M. *et al., Br. J. Rheumatol. 27*:372-374 (1988)).

[0181] Increase in warmth of overlying skin is a cardinal feature of inflammation and can be measured in various ways (*e.g.*, Bacon, P.A. *et al., Clin. Rheum. Dis. 2*:51-65 (1976)). Infrared quantitative thermography shows reproducible changes in disease activity and is useful in assessing efficacy of a treatment composition or method. Thermography provides a noninvasive, reproducible, sensitive, and quantifiable method of assessing improvement in joint inflammation (Ingpen, M.L., *Ann. Phys. Med. 9*:322-327 (1968)).

TABLE 3

Outcome Measures for Clinical Trials in Arthritis

Altman, R.D. *et al., Clin. Rheum. Dis. 9*:681-693 (1983)

FDA Guidelines (1977)	**Bellamy and Buchanan** *Clin. Rheumatol. 3*:293-305 (1984)
Joint swelling	Pain
Joint redness	Patient global assessment
Tenderness on pressure	Range of movement
Pain at rest or on motion	Physician global assessment
Range of motion	Joint stiffness
50-foot walking time	Qualitative aspects of sleep
Clinician's global assessment	Walking time
Patient's global assessment	Activities of daily living
Altman *et al.* *(supra)*	Joint tenderness
	Analgesic compound
Pain (using visual analogue scales)	Joint swelling
Tenderness on pressure/motion	Signal joints
Clinician's global assessment of current status and degree of change in status	Ascent time
Patient's global assessment of current status and degree of change in status	Muscle power**
50-foot walking time (for patients with hip and/or knee involvement)	Hand function
Grip strength (for patients with hand involvement)	Radiology
	Joint temperature

Laboratory Tests

[0182] Certain laboratory tests reflect the severity of joint inflammation and may be used to monitor the efficacy of the therapeutic compositions and methods of this invention. The most frequently used test is the erythrocyte sedimentation rate (ESR). Other measures used include evaluation of various acute-phase reactants, such as C-reactive protein, haptoglobin, fibrinogen, α-2 macroglobulin, and plasma viscosity (McConkey, B. *et al., Q.J. Med., New Series 41*:115-125 (1972); McConkey, B. *et al., Q.J. Med., New Series 42*:785-791 (1973); Constable, T.J. *et al., Lancet 1*:1176-1179 (1975); Crook, L. *et al., Ann. Clin. Lab. Sci. 10*:368-376 (1980); Dixon, J.A. *et al., Scand. J. Rheumatol. 13*:39-44 (1984); Cockel, R. *et al., Ann. Rheum. Dis. 30*:166-170 (1971)); titer of IgM rheumatoid factor or of immune complexes (Pope, R.M. *et al., Ann. Rheum. Dis. 45*:183-189 (1986); Reeback J.S. *et al, Ann. Rheum. Dis. 44*:79-82 (1986); Reynolds, W.J. *et al., J. Rheumatol. 13*:700-706 (1986)); tests of lymphocyte function (Reynolds, W.J. *et al., J. Rheumatol. 13*:700-706 (1986); Alepa, F.P. *et*

al., Arthritis Rheum. 13:754-760 (1970); Swanson, M.A. *et al., N. Engl. J. Med. 277*:163-170 (1967)); displacement of L-tryptophan from serum albumin; serum iron concentration (Cockel, *supra*), eosinophilia, thrombocytosis (Hutchinson, R.M. *et al., Ann. Rheum. Dis. 35*:138-142 (1976)); serum concentrations of sulfhydryl groups (Lorber, A. *et al., Metabolism 20*:446-455 (1971)); serum copper concentrations (Brown, D.H. *et al., Ann. Rheum. Dis. 38*:174-176 (1979)); serum propeptide levels (Horsley-Petersen *et al., Rheum. 29*:592-599 (1986)); synovial fluid analysis (Hall, S.H. *et al., Ann. Rheum. Dis. 37*:351-356 (1978)).

[0183] Various methods are used to score radiologic changes in rheumatoid arthritis, the most useful of which are count erosions and assessment of joint space narrowing. Radionuclides can also be used to quantify joint inflammation (Dick, W.C., *Semin. Arthritis Rheum. 1*:301-325 (1972); Wallace, D. J. *et al., Arthritis Rheum. 11*:172-176 (1981)). These are administered intra-articularly and the rate of clearance from the joint determined or, alternatively, they are administered iv and the rate of accumulation over a joint (or joints) measured. The clearance of ^{133}Xe after intra-articular injection provides an indirect measurement of synovial blood flow. $^{99m}TcO_4$ is also used. Radionuclide joint uptake in both large and small joints is known to be reduced with successful anti-rheumatic therapeutics such as NSAIDs, corticosteroids, gold or D-penicillamine.

RESULTS

[0184] According to the 8 measures listed under "FDA Guidelines" above, greater than 80% of the patients treated with the cannabinoid compounds described herein show significant cumulative improvement across all measures.

[0185] Having generally described preferred embodiments of the invention, the same will be more readily understood through reference to the following nonlimiting examples which are provided by way of illustration.

EXAMPLES

[0186] Unless otherwise stated, chemical reagents were purchased from commercial sources, for example Aldrich Chemical Company (Milwaukee, Wisconsin, USA) or Lancaster Synthetics Ltd, (Lancashire, UK). Temperatures are in degrees Celsius. Mass spectra were obtained on a Micromass ZQ 2000 benchtop electrospray mass spectrometer. Samples were dissolved in either

ethanol or a 1:1 mixture of acetonitrile:water containing 0.2% formic acid. Molecular species recorded in the positive ion mode are noted as M+H or M+Na, while those obtained in the negative ion mode are noted as M-H. NMR spectra were obtained on a Varian INOVA 500 Mhz nuclear magnetic resonance spectrometer or, where stated, a Varian INOVA 300 Nhz NMR spectrometer .

EXAMPLE 1

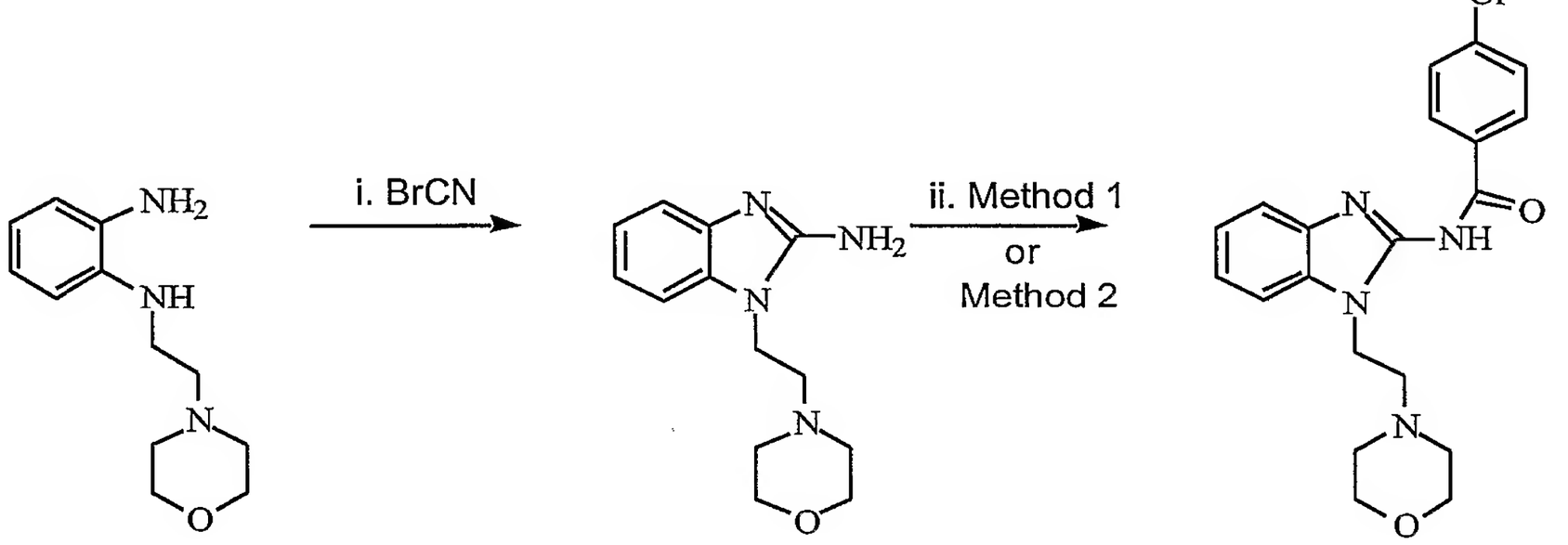
Preparation of 4-chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (1)

[0187] 2-Chloronitrobenzene (10.0 g, 63.5 mmol), 2-aminoethylmorpholine (17.5 ml, 133.5 mmol) and 1-butanol (20 ml) were combined and heated at 100°C for 16 hours under an atmosphere of nitrogen. Excess reagent and solvent were removed in vacuo and the residue diluted with EtOAc (200 ml) and 1M NaOH (100 ml). The organic phase was separated and the remaining aqueous solution extracted with EtOAc (2x100 ml). The organic extracts were combined and washed with brine (100 ml), dried over sodium sulfate and evaporated. The crude product was columned (silica) using a gradient from petroleum spirit to EtOAc (Rf 0.28, 50%EtOAc/Pet Spirit) to give the 2-morpholin-4-yl-ethyl)-(2-nitro-phenyl)-amine (15.6 g, 98% yield). ESMS 252 (M+H).

[0188] 2-Morpholin-4-yl-ethyl)-(2-nitrophenyl)-amine (7.5 g, 29.9 mmol) was dissolved in 50% aq EtOH (150 ml) and heated to 80°C under an atmosphere of nitrogen. To this was added sodium hydrosulfite dissolved in water and the mixture refluxed for 3h. If no color change occurs (incomplete reaction) more sodium hydrosulfite (10 g) was added (instant color change). The solvent was evaporated and 1M NaOH (100 ml) added and the mixture extracted with dichloromethane (DCM) (4x100 ml). The organic extracts were combined, washed with brine (100 ml), dried over sodium sulfate, filtered and evaporated. The crude product was columned (silica) using a gradient from DCM to 25%MeOH (methanol)/DCM (Rf 0.25, 10%MeOH/DCM) to give the title phenylenediamine (5.3 g, 80% yield). ESMS 222 (M+H).

[0189] *N*-(2-aminophenyl)-*N*-(2-morpholin-4-ylethyl)amine (3.7 g, 16.7 mmol) was dissolved in dry EtOH (ethanol) (40 ml). Cyanogen bromide (1.86 g,

17.6 mmol) was added and the mixture refluxed for 60 minutes. The reaction mixture was evaporated to dryness then diluted with brine (30 ml) and 5M NaOH (10 ml) and extracted with EtOAc (4x50 ml) . The combined EtOAc extracts were dried over sodium sulfate, filtered, evaporated and the crude residue columned (NEt_3 washed silica) using a gradient from DCM to 25%MeOH/DCM to give 1-(2-morpholin-4-yl-ethyl)-1*H*-benzoimidazol-2-ylamine (Rf 0.35, 10%MeOH/DCM). ESMS 247 (M+H)). ^{1}HNMR ($CDCl_3$, 500MHz) δ:7.42 (d, J = 7Hz, 1H), 7.14-7.04 (m 3H), 6.0 (bs, 2H), 4.03 (t, J = 4.5Hz), 2H), 3.72 (m, 4H), 2.76 (t, J = 4.5Hz, 2H), 2.58 (bs, 4H).



(1)

Method 1 (Kinetic):

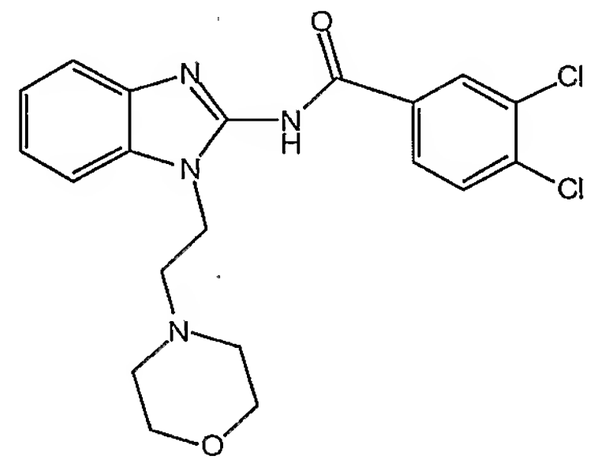
[0190] To a suspension of 60%NaH (0.194 g, 4.86 mmol) in dimethyl formamide **(DMF)** (10 ml) was added the 1-(2-morpholin-4-yl-ethyl)-1*H*-benzoimidazol-2-ylamine (1 g, 4.05 mmol) and the resulting mixture stirred at room temperature for 30 minutes. The reaction mixture was cooled to –50°C and 4-chlorobenzoyl chloride (0.51 ml, 4 mmol) added. The mixture was allowed to warm to room temperature over 60 minutes before diluting with EtOAc (100 ml) and washing with water (3x25 ml), brine (25 ml), drying over sodium sulfate and evaporating. The title compound **4-chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (1)** was precipitated from toluene/pet spirit to give a major component with identical Rf to the thermodynamic product.

[0191] ESMS (identical to above); NMR (identical to above); IR (identical to above).

Method 2 (Thermodynamic):

[0192] 1-(2-Morpholin-4-yl-ethyl)-1*H*-benzoimidazol-2-ylamine (2.4 g, 9.76 mmol) and N-methy morpholine (1.18 ml, 10.73 mmol) were dissolved in DMF (100 ml). To this was added 4-chlorobenzoyl chloride and the mixture stirred at 80°C for 6 hours. The solvent was evaporated and the title compound **4-chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (1)** was precipitated from hot EtOAc. (Rf 0.51, 100%EtOAc). ESMS 385/387 (M+H). ^{1}H-NMR ($CDCl_3$, 500MHz) δ: 12.38 (bs, 1H), 8.24 (d, J = 8.5Hz, 2H), 7.40 (d, J = 8.5Hz, 2H), 7.40-7.2 (m, 4H), 4.39 (t, J = 7Hz, 2H), 3.64 (m, 4H), 2.82 (t, J = 7Hz, 2H), 2.60 (bs, 4H). IR (cm^{-1}) 3306, 2949, 2815, 1571, 1552, 1528, 1485, 1398, 1351, 1334, 1303, 1150, 1117,1015,942,896, 766, 744, 665.

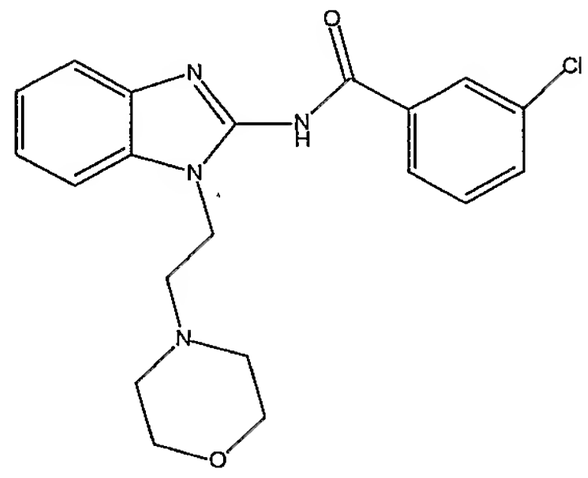
The following analogs were prepared in a similar fashion:



(2)

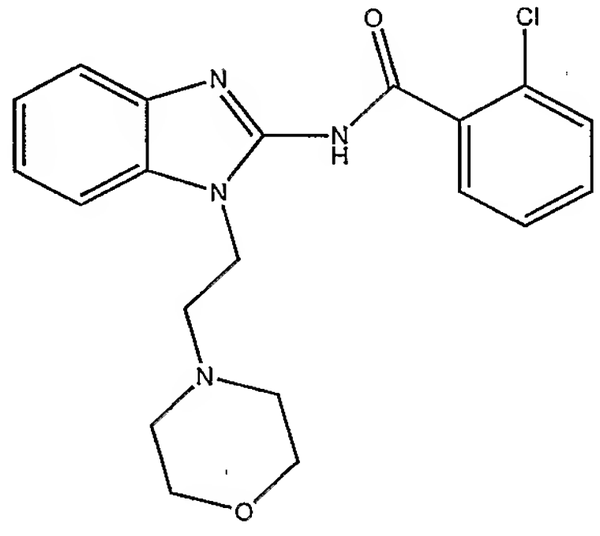
[0193] **3,4-Dichloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (2)** 1-(2-Morpholinoethyl)-1*H*-benzo[*d*]imidazol-2-amine (250 mg, 1.01 mmol) (described above) was combined with 3,4-dichlorobenzoyl chloride (211 mg, 1.01 mmol) in dichloromethane (10 ml) and allowed to react for 24 hours. The reaction mixture was treated with 2M sodium hydroxide (20 ml) and the phases separated. The aqueous phase was extracted with dichloromethane (20 ml) and the combined organic extracts washed with brine (20 ml). The combined organic extracts were then washed with water (20 ml), dried and the solvent removed *in vacuo*. The residue was purified by chromatography using a gradient from DCM to 30% MeOH/DCM to give **3,4-dichloro-*N*-(1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazole-2-yl)benzamide (2)** (370 mg, 87% yield). Rf 0.74 (5% EtOH/EtOAc). ESMS 418/420/422 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ:

9.43 (bs, 1H), 8.28 (m, 1H), 8.01 (m, 1H), 7.51 (d, J = 8.5Hz, 1H), 7.30 (m, 4H), 4.42 (t, J = 7.5Hz, 2H), 3.66 (t, J = 5Hz, 4H), 2.86 (t, J = 7.5Hz, 2H), 2.65 (bs, 4H).



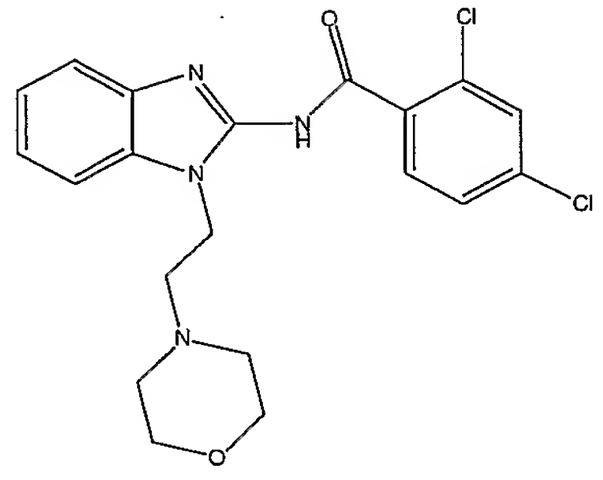
(3)

[0194] **3-Chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (3)** (314 mg, 81% yield) Rf 0.71 (5% EtOH/EtOAc). ESMS 385/387 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500 MHz) δ: 8.14 – 8.28 (m, 1H), 8.08 bs, 2H) 8.01 -7.97 (m, 2H), 7.59 - 7.55 (m, 1H), 7.54 – 7.46 (m, 1H), 7.44 – 7.36 (m, 2H), 7.32 – 7.26 (m, 1H), 4.71 (bs, 2H), 3.90 (bs, 4H), 3.23 (bs, 2H), 3.05 (bs, 4H).



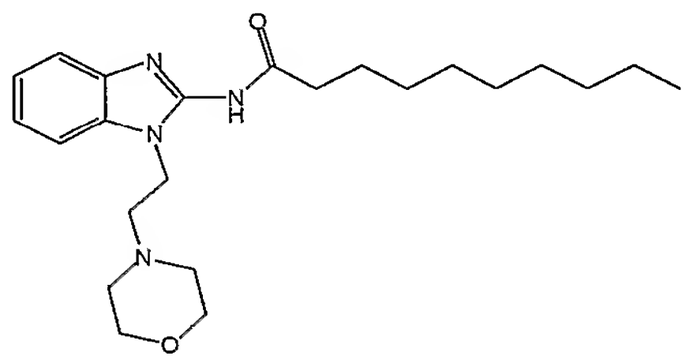
(4)

[0195] **2-Chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (4)** (150 mg, 38%) Rf 0.64 (5% EtOH/EtOAc). ESMS 385/387 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.89 (dd, J = 2.2, 7.8Hz, 1H), 7.84 (dd, J = 2.2, 7.8Hz, 1H), 7.31 (m, 6H), 4.49 (t, J = 6.0Hz, 2H), 3.73 (t, J = 4.0Hz, 4H), 3.0 (t, J = 6.0 Hz), 2.77 (bs, 4H).



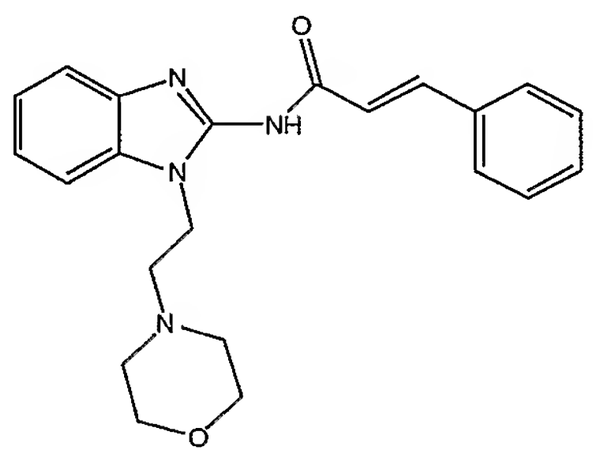
(5)

[0196] **2,4-Dichloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (5)** (320 mg, 76% yield) Rf 0.71 (5% EtOH/EtOAc). ESMS 418/422 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500 MHz) δ: 7.98-7.87 (m, 2H), 7.48 – 7.42 (m, 1H), 7.37 – 7.25 (m, 4H), 4.50 (bs, 2H), 3.55 (bs, 4H), 2.95 (bs, 2H), 2.72 (bs, 4H).



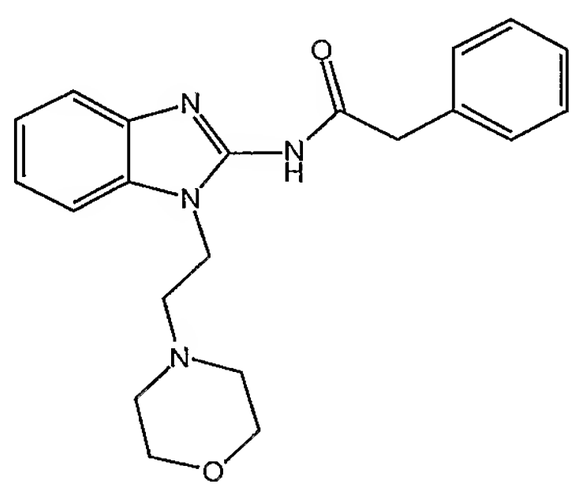
(6)

[0197] ***N*-(1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazole-2-yl)decanamide (6)** (310 mg, 76% yield) Rf (5% EtOH/EtOAc). ESMS 401 ($M+H^+$). ^{1}Hnmr (CDCl3, 500MHz) δ: 7.36 – 7.23 (m, 4H), 4.38 (bs, 2H), 3.75 (bs, 4H), 2.87 (bs, 2H), 2.68 (bs, 4H), 2.35 – 2.29 (m, 2H), 1.73 – 1.60 (m, 2H), 1.38 – 1.27 (m, 15H).



(7)

[0198] ***N*-(1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazole-2-yl)cinnamamide (7)** (250 mg, 66%), Rf (5% EtOH/EtOAc). ESMS 377 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.78-7.70 (m, 1H), 7.59-7.48 (m, 3H).7.42 – 7.26 (m, 5H), 6.82 – 6.79 (m, 1H), 6.48 – 6.45 (m, 1H), 4.40 (bs, 2H), 3.72 (bs, 4H), 2.85 (bs, 2H), 2.66 (bs, 4H).

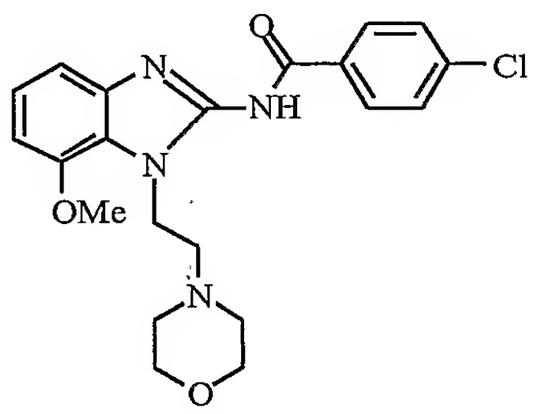


(8)

[0199] **2-phenyl-*N*-(1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazole-2-yl)acetamide (8)** (305 mg, 81% yield) Rf (5% EtOH/EtOAc). ESMS 365 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.56 – 7.1 (m, 9H), 4.40 (bs, 2H), 3.80 (bs, 4H), 3.78 (bs, 2H), 2.90 (bs, 2H), 2.71 (bs, 4H).

EXAMPLE 2

Preparation of 4-chloro-*N*-[7-methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (2)



(9)

[0200] 3-Nitrophenol (10 g, 72 mmol) was dissolved in hot concentrated hydrochloric acid (100 ml) and cooled rapidly (ice-brine bath) to give a fine suspension. Potassium chlorate (8.8 g, 72 mmol) was dissolved in water (100 ml) and added to the mixture and stirring continued at 0°C for 2 hours. The reaction

mixture was extracted with ethyl acetate (2x100 ml) and the combined organic extracts washed with water (100 ml), brine (100 ml), dried over sodium sulfate and evaporated in vacuo (water bath temp <45°C) to give 2-chloro-3-nitrophenol (not purified) (Rf 0.38, 33%EtOAc/Pet spirit). The crude residue was dissolved in DMF and to this was added potassium carbonate (19.3 g, 140 mmol) and methyl iodide (5.6 ml, 90 mmol). The reaction mixture was stirred overnight at room temperature before diluting with ethyl acetate (200 ml) and washing with water (4x100 ml) and brine (100 ml). The organic phase was dried over sodium sulfate to give 2-chloro-3-nitroanisole (not purified) (Rf 0.61. 33% EtOAc/Pet spirit). The crude residue obtained was dissolved in 2-aminoethylmorpholine 15.7 ml, 120 mmol) and butanol (50 ml) and heated at 100°C for 16 hours under an atmosphere of nitrogen. Excess reagent and solvent were removed in vacuo and the residue diluted with EtOAc (200 ml) and 1M NaOH (100 ml). The organic phase was separated and the remaining aqueous solution extracted with EtOAc (2x100 ml). The organic extracts were combined and washed with brine (100 ml), dried over sodium sulfate, filtered and evaporated. The crude product was columned (silica) using a gradient from petroleum spirit to EtOAc (Rf 0.17, 50%EtOAc/Pet Spirit) to give 2-methoxy-*N*-(2-morpholinoethyl)-6-nitrobenzenamine (7.0 g, 35% yield over 3 steps). ESMS 282 (M+H).

[0201] 2-Methoxy-*N*-(2-morpholinoethyl)-6-nitrobenzenamine (5 g, 17.8 mmol) was dissolved in ethanol (100 ml), 10%Pd-C (100 mg) added and the mixture hydrogenated at 20 psi for 6 hours. The catalyst was removed by filtration through celite and the solvent removed in vacuo. The crude material was columned (silica) using a gradient from DCM to 25%MeOH/DCM to give the 6-methoxy-*N'*-(2-morpholinoethyl)benzene-1,2-diamine (Rf 0.38, 10%MeOH/DCM). ESMS 252 (M+H). This compound (3.8 g, 15.2 mmol) was dissolved in dry EtOH (100 ml), cyanogen bromide (1.58 g, 15.2 mmol) was added and this mixture was refluxed for 60 minutes. The reaction mixture was evaporated to dryness then diluted with brine (30 ml) and 5M NaOH (10 ml) and extracted with EtOAc (4x50 ml) followed by extraction with BuOH (2x50 ml). The combined EtOAc extracts were dried over sodium sulfate and the crude residue precipitated from ethyl acetate /petroleum spirit to give 7-methoxy-1-(2-morpholinoethyl)-1*H*-benzo[d]imidazol-2-amine (Rf 0.15, 10%MeOH/DCM).

ESMS 277 (M+H). ^{1}HNMR ($CDCl_3$, 500MHz) δ:7.07 (d, J = 8Hz, 1H), 7.01 (dd, J = 8Hz, 8Hz, 1H), 6.57 (d, J = 8Hz, 2H), 6.0 (bs, 2H), 4.4 (m, 2H), 3.89 (s, 3H), 3.71 (m, 4H), 2.80 (m, 2H), 2.57 (bs, 4H). Yield 2.2g (53%). This compound (300 mg, 1.09 mmol) was dissolved in DMF (5 ml) and *N*-methylmorpholine (131 µl, 1.19 mmol). To this was added 4-chlorobenzoyl chloride (152 µl, 1.19 mmol) and the resulting mixture stirred at 50°C for 3 hours. The solvent was evaporated and the crude residue precipitated from hot EtOAc to provide the title compound, **4-chloro-*N*-[7-methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (9)** as a white solid. (Rf 0.51, 100%EtOAc). ESMS 415/417 (M+H). ^{1}HNMR ($CDCl_3$, 500MHz) δ: 12.42 (bs, 1H), 8.24 (d, J = 8.5Hz, 2H), 7.39 (d, J = 8.5Hz, 2H), 7.16 (dd. J = 8Hz, 8.5Hz, 1H), 6.95 (d, J = 8Hz, 2H), 6.77 (d, J = 8.5Hz, 1H), 4.61 (t, J = 7Hz, 2H), 3.98 (s, 3H), 3.63 (m, 4H), 2.80 (m, 2H), 2.60 (bs, 4H).

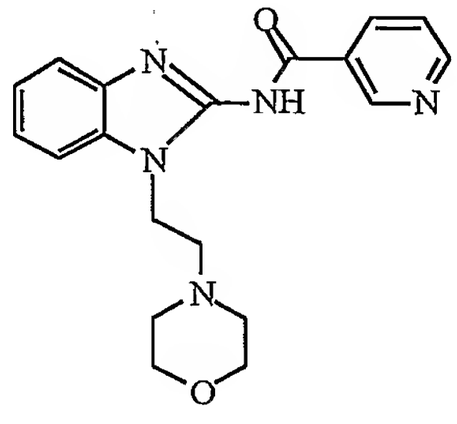
EXAMPLE 3

Preparation of *N*-[7-hydroxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]nicotinamide (10) and 3,5-ditrifluoromethyl-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (11)

General Method (from the corresponding acid):

[0202] The benzoic acid (2.03 mmol) was dissolved in 0.5 M *O*-benzotriazol-1-yl-*N,N,N',N'*,-tetramethyluronium hexafluorophosphate (HBTU)/DMF solution and N-methyl morpholine (231 µl, 2.1 mmol) added. This was stirred for 5 minutes before adding the substituted benzimidazole (500 mg, 2.03 mmol) dissolved in DMF (5 ml) and the mixture stirred at 50°C for 6 hours. The reaction mixture was diluted with ethyl acetate (100 ml), washed with 1M sodium hydroxide (50 ml), water (50 ml), brine (50 ml), dried over sodium sulfate and evaporated. The crude residue was either precipitated or columned as required.

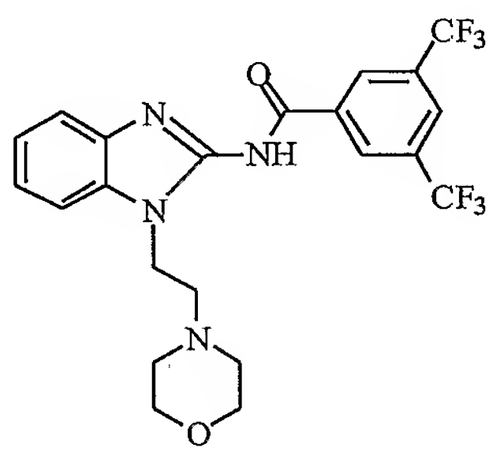
Preparation of *N*-[7-hydroxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]nicotinamide (3)



(10)

[0203] Following the general method described above the ***N*-[7-hydroxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]nicotinamide (10)** was precipitated from ethyl acetate/Pet spirit (Rf 0.48, 10%MeOH/DCM) as a pale yellow solid (365mg, 51% yield). ESMS 352 (M+H). ^{1}HNMR ($CDCl_3$, 500MHz) δ: 12.32 (bs, 1H), 9.52 (m, 1H), 8.71 (m, 1H), 8.52 (m, 1H). 7.40-7.28 (m, 5H), 4.40 (m, 2H), 3.64 (m, 4H), 2.83 (m, 2H), 2.60 (bs, 4H).

3,5-ditrifluoromethyl-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (4)

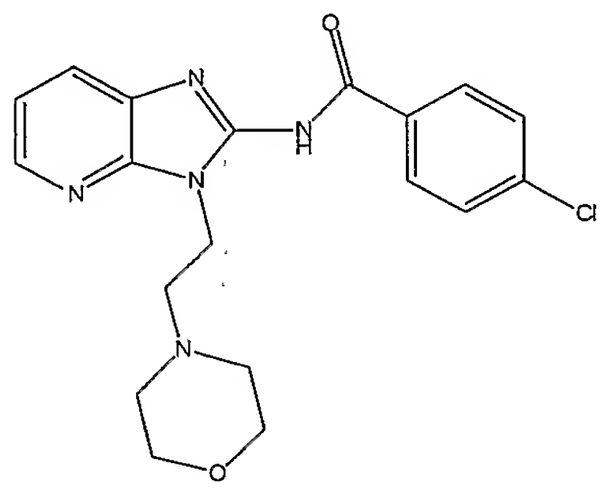


(11)

[0204] Following the general procedure described above, the **3,5-ditrifluoromethyl-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide (11)** was purified by column chromatography using Pet spirit/EtOAc gradient (Rf 0.71, 100%EtOAc) to give the desired compound as a pale yellow solid (520mg, 52% yield). ESMS 487 (M+H).). ^{1}HNMR ($CDCl_3$, 500MHz) δ: 12.29 (bs, 1H), 8.77 (s, 2H), 7.98 (s, 1H), 7.41-7.30 (m, 4H), 4.42 (t, J = 7Hz, 2H), 3.65 (m, 4H), 2.84 (t, J = 7Hz, 2H), 2.62 (bs, 4H).

EXAMPLE 4

4-Chloro-*N*-(3-(2-morpholinoethyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzamide (12)



(12)

[0205] 2-Chloro-3-nitropyridine (10.0 g, 63.5 mmol), 2-aminoethylmorpholine (17.5 ml, 133.5 mmol) and 1-butanol (20 ml) were combined and heated at 100°C for 16 hours under an atmosphere of nitrogen. Excess reagent and solvent were removed in vacuo and the residue diluted with EtOAc (200 ml) and 1M NaOH (100 ml). The organic phase was separated and the remaining aqueous solution extracted with EtOAc (2 x 100 ml). The organic extracts were combined and washed with brine (100 ml), dried over sodium sulfate and evaporated. The crude product was columned (silica) using a gradient from petroleum spirit to EtOAc (Rf 0.28, 50% EtOAc/Pet Spirit) to give N^2-(2-morpholinoethyl)-2-nitrobenzenamine (15.6 g, 98% yield). ESMS 253 ($M+H^+$).

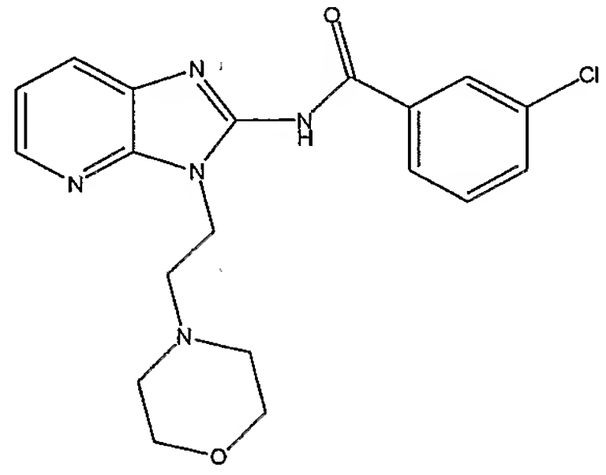
[0206] N^2-(2-morpholinoethyl)-3-nitropyridine-2-amine (3.12 g, 12.4 mmol) was dissolved in EtOH (120 ml) and combined with 10% Pd-C (399 mg). The resulting mixture was hydrogenated at 20 psi until consumption of hydrogen gas had ceased. The mixture was filtered through celite to remove the catalyst and the filtrate evaporated. The solvent was removed *in vacuo* to give N^2-(2-morpholinoethyl)pyridine-2,3-diamine (2.6 g, 95% yield) which was used without further purification. ESMS 223 ($M+H^+$).

[0207] N^2-(2-morpholinoethyl)pyridine-2,3-diamine (1.5 g, 6.75 mmol) was dissolved in dry MeOH (20 ml) and the reaction mixture cooled to $0^{O}C$. Cyanogen bromide (0.785 g, 7.41 mmol) in MeOH (15 mL) was added and the reaction mixture stirred at $0^{O}C$ for 3 hours followed by room temperature

overnight. A further quantity of cyanogen bromide (0.30 g, 2.83 mmol) was added and the reaction mixture stirred at room temperature for 5 hours. The reaction mixture was evaporated to dryness then diluted with brine (30 ml) and 5M NaOH (10 ml) and extracted with dichloromethane (3 x 50 ml). The combined dichloromethane extracts were dried over sodium sulfate, filtered, evaporated and the crude residue purified by crystallization from EtOAc/Pet spirit (60-80^{O}C) to give 3-(2-morpholinoethyl)-3H-imidazo[4,5-*b*]pyridin-2-amine (1.4 g, 84% yield). Rf (5% EtOH/EtOAc). ESMS 176 ($M+H^{+}$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.99 (d, J= 5Hz, 1H), 7.55 (d, J = 7.5Hz, 1H), 4.95 (m, 2H), 4.88 (dd, J = 5.75 Hz, 1H), 3.75 (bs, 4H), 2.77 (m, 2H), 2.61 (bs, 4H).

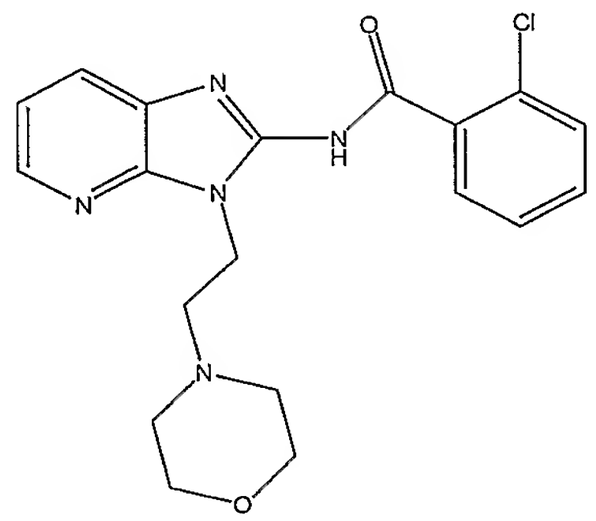
[0208] To a solution of 4-chlorobenzoyl chloride (117 mg, 0.67 mmol) in dichloromethane (10 mL) was added 3-(2-morpholinoethyl)-3H-imidazo[4,5-*b*]pyridin-2-amine (149 mg, 0.603 mmol) and the reaction mixture stirred at room temperature for 10 hours. The reaction mixture was diluted with dichloromethane (20 mL) and the organic phase washed with aqueous sodium hydroxide (2M, 20 mL) followed by water (20 mL). The combined aqueous washings were extracted with dichloromethane (20 mL) and the combined organic extracts were dried and the solvent removed *in vacuo*. The residue was purified by chromatography on a gradient using DCM to MeOH to give **4-chloro-*N*-(3-(2-morpholinoethyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzamide (12)** (203 mg, 87% yield) Rf (5% EtOH/EtOAc). ESMS 386/388 ($M+H^{+}$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 8.25 (m, 3H), 7.56 (d, J = 8.6Hz, 1H), 7.20 (m, 2H), 7.18 (m, 1H), 4.52 (t, J = 6.7Hz, 2H), 3.60 (bs, 4H), 2.96 (t, J = 6.7Hz, 2H), 2.65 (bs, 4H).

The following analogs were prepared in a similar fashion:



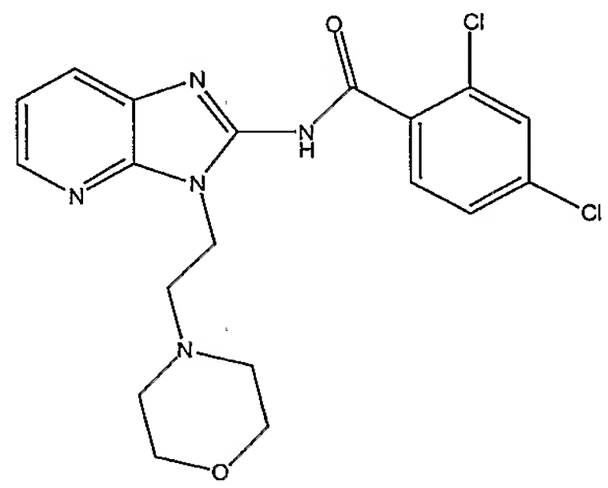
(13)

[0209] **3-chloro-*N*-(3-(2-morpholinoethyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzamide (13)** (110 mg, 53% yield) Rf (5% EtOH/EtOAc). ESMS 386/388 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 8.28 – 8.22 (m, 1H), 8.21 – 8.17 (m, 1H), 7.62 – 7.58 (m, 1H), 7.49 – 7.45 (m, 1H), 7.42 – 7.46 (m, 1H), 7.24 – 7.19 (m, 1H).



(14)

[0210] **2-chloro-*N*-(3-(2-morpholinoethyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)benzamide (14)** (120 mg, 60% yield) Rf (5% EtOH/EtOAc). ESMS 386/388 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 8.07 (bd, J = 5.0Hz), 1H), 7.76 (m, 1H), 7.62 (m, 2H), 7.39 (m, 1H), 7.28 (m, 1H), 7.02 (m, 1H), 4.29 (m, 2H), 3.76 (bs, 4H), 2.83 (m, 2H), 2.66 (bs, 4H).

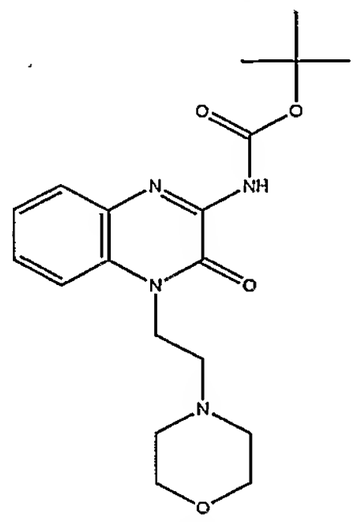


(15)

[0211] **2,4-dichloro-N-(3-(2-morpholinoethyl)-3H-imidazo[4,5-b]pyridin-2-yl)benzamide (15)** (140 mg, 59% yield) Rf (5% EtOH/EtOAc). ESMS 418/420/422 (M+H+). 1Hnmr (CDCl3, 500MHz) δ: 8.07 – 8.04 (m, 2H), 7.63 – 7.58 (m, 2H), 7.10 – 7.05 (m, 2H), 4.31 – 4.26 (m, 2H), 3.75 (bs, 4H), 2.85 – 2.79 (m, 2H), 2.65 (bs, 4H).

EXAMPLE 5

Synthesis of *tert*-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate (16).



(16)

[0212] 2-Chloronitrobenzene (10.0 g, 63.5 mmol), 2-aminoethylmorpholine (17.5 ml, 133.5 mmol) and 1-butanol (20 ml) were combined and heated at 100°C for 16 hours under an atmosphere of nitrogen. Excess reagent and solvent were removed in vacuo and the residue diluted with EtOAc (200 ml) and 1M NaOH (100 ml). The organic phase was separated and the remaining aqueous solution extracted with EtOAc (2x100 ml). The organic extracts were combined and washed with brine (100 ml), dried over sodium sulfate and evaporated. The crude product was columned (silica) using a gradient from petroleum spirit to EtOAc (Rf 0.28, 50%EtOAc/Pet Spirit) to give the 2-(morpholin-4-yl)-ethyl-(2-nitrophenyl)amine (15.6 g, 98% yield). ESMS 252 (M+H).

[0213] 2-(Morpholin-4-yl)-ethyl-(2-nitrophenyl)amine (7.5 g, 29.9 mmol) was dissolved in 50% aqueous EtOH (150 ml) and heated to 80°C under an atmosphere of nitrogen. To this was added sodium hydrosulfite dissolved in water and the mixture refluxed for 3 hours. If no color change occurred (incomplete reaction) more sodium hydrosulfite (10 g) was added (until complete color change). The solvent was evaporated and 1M NaOH (100 ml) added and the mixture extracted with DCM (4x100 ml). The organic extracts were combined, washed with brine (100 ml), dried over sodium sulfate, filtered and evaporated. The crude product was columned (silica) using a gradient from DCM to 25%MeOH/DCM (Rf 0.25, 10%MeOH/DCM) to give the desired

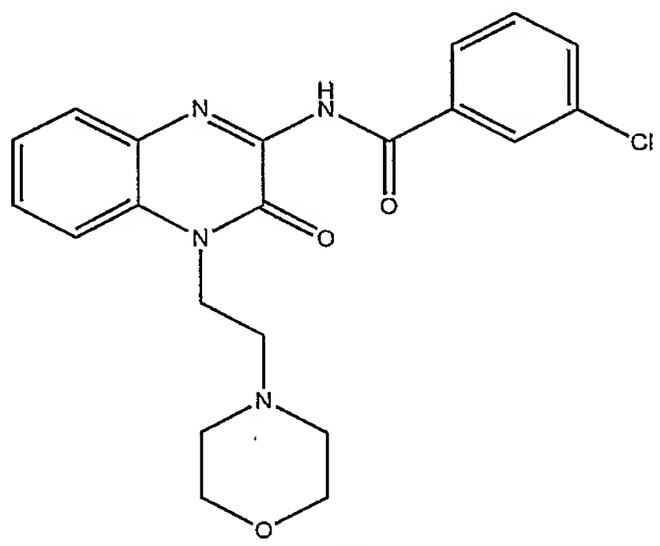
phenylenediamine, *N*-(2-aminophenyl)-*N*-(2-morpholin-4-ylethyl)amine (5.3 g, 80% yield). ESMS 222 (M+H).

[0214] *N*-(2-aminophenyl)-*N*-(2-morpholin-4-ylethyl)amine (5.4 g, 24 mmol) and diethyloxomalonate (prepared according to Pardo S.N. and Salomon R.G., *J. Org. Chem*. 1981, 46, 2598-2599) (3.72 ml, 24 mmol) were dissolved in toluene and refluxed, while removing water under Dean-Stark conditions, for 16 hours. After cooling the solvent was evaporated and the residue columned (silica) using a gradient from EtOAc to 10% EtOH/EtOAc (Rf 0.30, 5% EtOH/EtOAc) to give ethyl 3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxylate (5.7 g, 72% yield). ESMS 332 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.96 (dd, J = 1.5, 8 Hz, 1H), 7.66 (m, 1H), 7.40 (m, 2H), 4.51 (q, J = 7.5 Hz, 2H), 4.43 (t, J = 7.0 Hz, 2H), 3.70 (m, 4H), 2.71 (t, J = 7.0 Hz, 2H), 2.59 (bm, 4H), 1.45 (t, J = 7.5 Hz, 3H).

[0215] Ethyl 3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxylate (1.55 g, 4.7 mmol) was dissolved in ethanol (20 ml) to which was added 1M NaOH (4.8 ml, 4.8 mmol). The reaction mixture was stirred at 50°C for 3 hours, cooled and evaporated to dryness to give the sodium salt of 3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxylic acid ESMS 304 ($M+H^+$) which was suspended in tert-butanol (50 ml) together with diphenylphosphoryl azide (4 ml, 18.8 mmol) and the resulting mixture was refluxed under an atmosphere of nitrogen for 24 hours. Upon cooling the solvent was evaporated and the crude residue dissolved in EtOAc (100 ml), washed with 1M NaOH (2 x 50 ml), brine (50 ml), dried over sodium sulfate and evaporated. The crude residue was columned (silica pre-washed with 1% NEt_3/EtOAc) using a gradient from EtOAc to 20% EtOH/EtOAc to give the desired product ***tert*-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate (16)** (600 mg, 34% yield, Rf 0.44 (5% EtOH/EtOAc)), ESMS 375 ($M+H^+$), ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 8.53 (bs, 1H), 7.89 (dd, J = 1.5, 8 Hz, 1H), 7.46-7.41 (m, 1H), 7.36-7.30 (m, 2H), 4.45 (t, J = 7 Hz, 2H), 3.70 (m, 4H), 2.71 (t, J = 7.5 Hz, 2H), 2.58 (bs, 4H), 1.56 (s, 9H) and the deprotected compound 3-amino-1-(2-morpholinoethyl)quinoxalin-2(1*H*)-one (500 mg, 38% yield, Rf 0.31 (5% EtOH/EtOAc)), ESMS 275 ($M+H^+$).

EXAMPLE 6

Synthesis of 3-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (17), *N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)-1-naphthamide (18), 2-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (19), 4-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (20), and 3,4-dichloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (21).

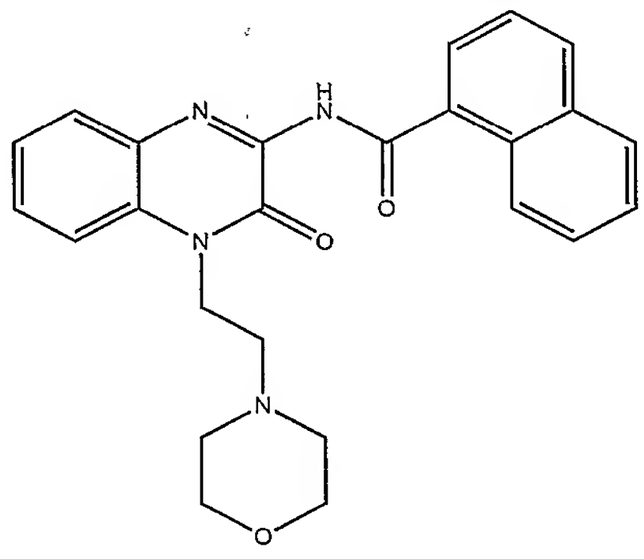


(17)

[0216] 3-Chloro-1-(2-morpholinoethyl)quinoxalin-2(1*H*)-one (5 g, 17 mmol) was dissolved in dry toluene (50 ml) and to this was added 50% w.v. sodium amide in toluene (approx. 1.7 ml, 21mmol). The resulting mixture was stirred at reflux for 5 hours, cooled and carefully quenched with 25% aqueous ethanol (10 ml). The mixture was diluted with EtOAc (200 ml) and washed with 1M sodium hydroxide (2 x 50 ml), brine (50 ml), dried over sodium sulfate, filtered and evaporated. The crude residue was columned (silica) using a gradient from EtOAc to 50% EtOH/EtOAc to give 3-amino-1-(2-morpholinoethyl)quinoxalin-2(1*H*)-one (3.3 g, 72% yield) (Rf 0.29, 5% EtOH/EtOAc). ESMS 275 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 7.49 (m, 1H), 7.28 (m, 3H), 5.75 (bs, 2H), 4.45 (t, J = 8.0Hz, 2H), 3.74 (m, 4H), 2.72 (t, J = 8.0Hz, 2H), 2.62 (bs, 4H). This compound was also made by dissolving *tert*-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate (684 mg, 1.83 mmol) in trifluoroacetic acid (5 ml), stirring the reaction mixture for 30 min and evaporating the reaction to dryness. The crude residue was dissolved in EtOAc (100 ml), washed with 1M NaOH (50 ml), brine (50 ml), dried over sodium sulfate and evaporated to give 3-amino-1-(2-morpholinoethyl)quinoxalin-2(1*H*)-one identical with that prepared by the method immediately above.

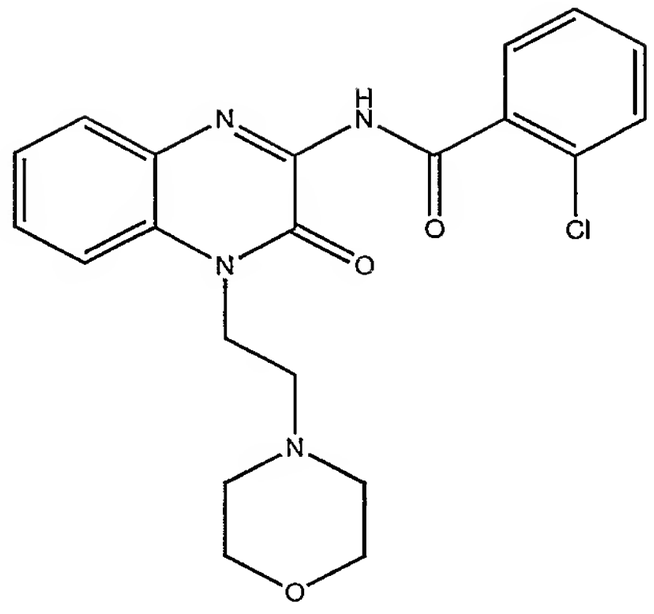
[0217] 3-Amino-1-(2-morpholinoethyl)quinoxalin-2(1*H*)-one (500 mg, 1.82 mmol) was dissolved in dry pyridine (20 ml) and 3-chlorobenzoyl chloride (254 µl, 2 mmol) added and the reaction mixture was heated at 50°C for 2 hours. Upon cooling the solvent was evaporated and the crude residue dissolved in EtOAc (100 ml), washed with 1M NaOH (50 ml), brine (50 ml), dried over sodium sulfate and evaporated. The crude residue was columned (silica) using a gradient from EtOAc to 20% EtOH/EtOAc to give **3-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (17)** (437 mg, 58% yield). Rf 0.39 (5% EtOH/EtOAc). ESMS 413/415 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.85 (bs, 1H), 7.97 (m, 2H), 7.86 (m, 1H), 7.60-7.38 (m, 5H), 4.52 (bs, 2H), 3.74 (bs, 4H), 2.77 (bs, 2H), 2.62 (bs, 4H).

[0218] The following analogs were prepared in a similar fashion:



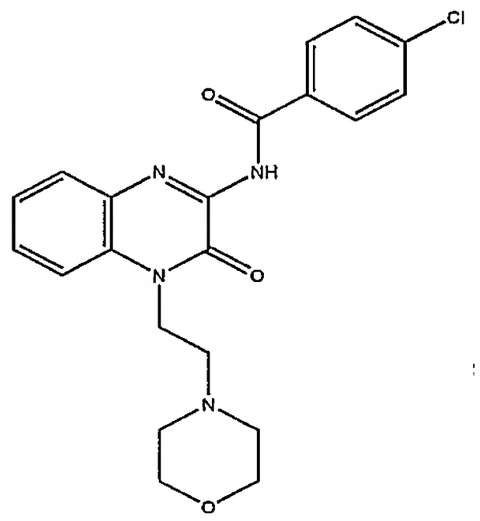
(18)

***N*-(1,2-Dihydro-1-(2-morpholinetheyl)-2-oxoquinoxalin-3-yl)-1-napthamide (18)** (27% yield). Rf 0.40 (10% EtOH/EtOAc). ESMS 429 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 300MHz) 8.36 – 8.30 (m, 1H), 7.93 – 7.79 (m, 4H), 7.78 – 7.60 (m, 1H), 7.47 - 7.18(m, 5H), 4.38 (bs, 2H), 3.61 (bs, 4H), 2.64 (bs, 2H), 2.49 (bs, 4H).



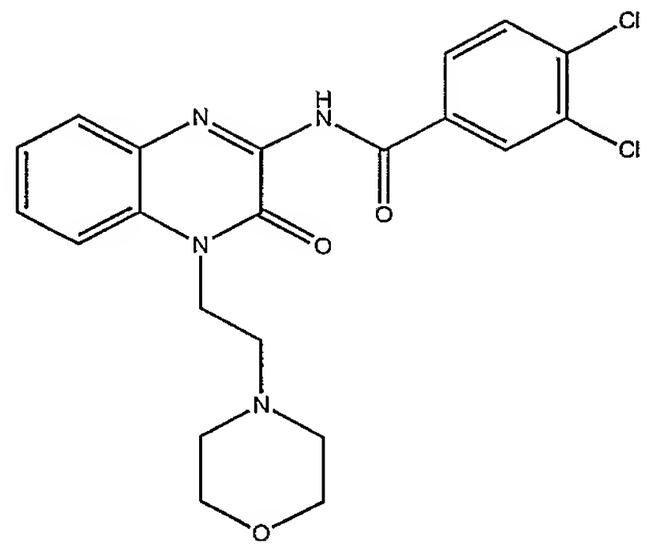
(19)

[0219] **2-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (19)** (287 mg, 38% yield). Rf 0.38 (5% EtOH/EtOAc). ESMS 413/415 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.86 (bs, 1H), 7.88-7.79 (m, 2H), 7.59-7.30 (m, 6H), 4.49 (bs, 2H), 3.71 (bs, 4H), 2.75 (bs, 2H), 2.60 (bs, 4H).



(20)

[0220] **4-chloro-*N*-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide (20)** (535 mg, 71% yield). Rf 0.32 (5% EtOH/EtOAc). ESMS 413/415 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.86 (bs, 1H), 7.95 (m, 3H), 7.50 (m, 3H), 7.40 (m, 2H), 4.50 (t, J = 7 Hz, 2H), 3.72 (m, 4H), 2.75 (t, J = 7.5 Hz, 2H), 2.61 (bs, 4H).

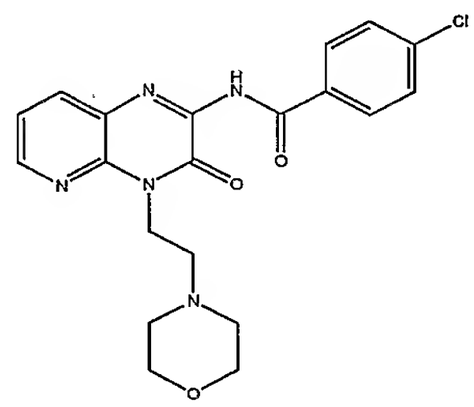


(21)

[0221] **3,4-Dichloro-*N*-(1,2-dihydro-1-(2-morpholinetheyl)-2-oxoquinoxalin-3-yl)-benzamide (21)** (33% yield). Rf 0.42 (10% EtOH/EtOAc). ESMS 447/449 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 300MHz) δ: 7.79 (bs, 1H), 7.52-7.30 (m, 6H), 4.49 (bs, 2H), 3.62 (bs, 4H), 2.63 (bs, 2H), 2.50 (bs, 4H).

EXAMPLE 7

Synthesis of 4-chloro-*N*-(3,4-dihydro-4-(2-morpholinoethyl)-3-oxopyrido [3,2-*b*]pyrazin-2-yl)benzamide (22)



(22)

[0222] 2-Chloro-3-nitropyridine (10.0 g, 63.5 mmol), 2-aminoethylmorpholine (17.5 ml, 133.5 mmol) and 1-butanol (20 ml) were combined and heated at 100°C for 16 hours under an atmosphere of nitrogen. Excess reagent and solvent were removed *in vacuo* and the residue diluted with EtOAc (200 ml) and 1M NaOH (100 ml). The organic phase was separated and the remaining aqueous solution extracted with EtOAc (2 x 100 ml). The organic extracts were combined and washed with brine (100 ml), dried over sodium sulfate and evaporated. The crude product was columned (silica) using a gradient from petroleum spirit to EtOAc (Rf 0.28, 50% EtOAc/Pet Spirit) to give N^2-(2-morpholinoethyl)-3-nitropyridine-2-amine (15.6 g, 98% yield). ESMS 253 (M+H).

[0223] N^2-(2-morpholinoethyl)-3-nitropyridine-2-amine (3.12 g, 12.4 mmol) was dissolved in EtOH (120 ml) and combined with 10% Pd-C (399 mg). The resulting mixture was hydrogenated at 20 psi until consumption of hydrogen gas had ceased. The mixture was filtered through celite to remove the catalyst and the filtrate evaporated. The solvent was removed *in vacuo* to give N^2-(2-morpholinoethyl)pyridine-2,3-diamine (2.6 g, 95% yield) which was used without further purification. ESMS 223 (M+H).

[0224] N^2-(2-morpholinoethyl)pyridine-2,3-diamine (2.20 g, 9.92 mmol) was combined with oxalic acid (1.38 g, 10.91 mmol) in aqueous hydrochloric acid (5M, 11 mL) and heated at reflux for 16 hours. The solvent was removed *in vacuo* and the solid residue added portion-wise to phosphorus oxychloride (32.9 g,

210 mmol) then heated at reflux for 5 hours. Once cooled the reaction mixture was gradually poured onto ice then treated with aqueous sodium hydroxide (5M) to pH 13 followed by the addition of brine (50 ml). The precipitated salt was removed by filtration and the aqueous phase extracted with ethyl acetate (2 x 200 ml), dried and the solvent removed *in vacuo* to give 2-chloro-4-(2-morpholinoethyl)pyrido[3,2-*b*]pyrazin-3-(4*H*)-one (1.0 g, 34%). ESMS 294/296 (M+H).

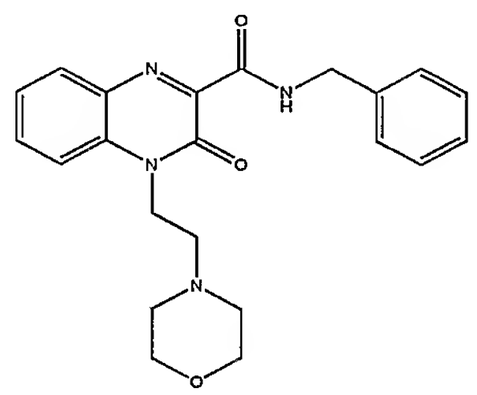
[0225] 2-Chloro-4-(2-morpholinoethyl)pyrido[3,2-*b*]pyrazin-3-(4*H*)-one (0.85 g, 2.89 mmol) was combined with dry formamide (10 ml) in thoroughly dry glassware, under nitrogen, and heated to 160° over 30 minutes then kept at 160° for 3 hours. The reaction mixture was allowed to cool before dissolving in ethyl acetate (50 ml). The organic phase was washed with a 1:1 mixture of aqueous sodium hydroxide and brine (2 x 50 ml). The combined aqueous washings were extracted with ethyl acetate (50 ml) and the combined organic extracts dried and the solvent removed *in vacuo* to give 2-amino-4-(2-morpholinoethyl)pyrido[3,2-*b*]pyrazin-3-(4*H*)-one (420 mg, 53%). ESMS 276 ($M+H^+$).

[0226] 2-Amino-4-(2-morpholinoethyl)pyrido[3,2-*b*]pyrazin-3-(4*H*)-one (420 mg, 1.53 mmol) was combined with 4-chlorobenzoyl chloride (267 mg, 1.53 mmol) in pyridine (10 ml) and heated at 70° for 3 hours. A further portion of 4-chlorobenzoyl chloride (27 mg, 0.15 mmol) was added and the reaction mixture heated at 85° for 2 days. A further portion of 4-chlorobenzoyl chloride (230 mg, 1.31 mmol) was added and the reaction mixture heated at 85° for a further 10 hours. The reaction mixture was allowed to cool then diluted with water (30 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were dried and the solvent removed *in vacuo*. The residue was partially purified by chromatography using a gradient from 100% dichloromethane to 100% methanol, the desired fractions combined and the solvent removed *in vacuo*. The residue was recrystallized from toluene/diethyl ether to give **4-chloro-*N*-(3,4-dihydro-4-(2-morpholinoethyl)-3-oxopyrido [3,2-*b*]pyrazin-2-yl)benzamide (22)** (420 mg, 66% yield). Rf 0.4 (5% EtOH/EtOAc). ESMS 414/416 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.92 (bs, 1H), 8.49 (dd, J = 2.0, 4.5Hz, 1H), 8.24 (dd, J = 2.0, 8.0Hz, 1H), 7.95 (dt, J = 2.5, 8.5Hz, 2H), 7.52 (dt, J = 2.5, 9.5Hz, 2H), 7.36 (dd, J = 5,

8.5Hz, 1H), 4.73 (t, J = 6.5Hz, 2H), 3.65 (bt, J = 4.5Hz, 4H), 2.80 (t, J = 6Hz, 2H), 2.60 (bs, 4H).

EXAMPLE 8

Synthesis of *N*-benzyl-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide (23), 3,4-dihydro-4-(2-morpholinoethyl)-3-oxo-*N*-phenethylquinoxaline-2-carboxamide (24), and *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide (25).



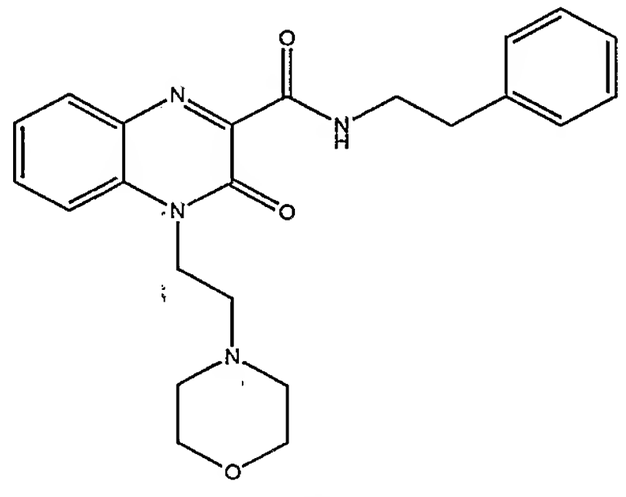
(23)

[0227] Ethyl 3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxylate (prepared as in Example 1) (500 mg, 1.51 mmol) was dissolved in ethanol (10 ml) to which was added 1M NaOH (1.75 ml, 1.75 mmol). The reaction mixture was stirred at 50°C for 3 hours, cooled and evaporated to dryness to give the sodium salt of 3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxylic acid which was used without further purification. ESMS 304 ($M+H^+$).

[0228] This compound (approx 1.51 mmol) was dissolved in DMF (10 ml) to which was added BOP reagent (benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexaflurophosphate) (685 mg, 1.55 mmol) and *N*-methylmorpholine (170 µl, 1.55 mmol). The resulting solution was stirred for 5 min before the addition of benzylamine (174 µl, 1.6 mmol). The reaction mixture was stirred for 2 hours, diluted with EtOAc (100 ml), washed with 1M sodium hydroxide (2 x 50 ml), water (50 ml), brine (50 ml), dried over sodium sulfate, filtered and evaporated. The crude residue was columned (silica) using a gradient from EtOAc to 25%EtOH/EtOAc (Rf 0.16, 5%EtOH/EtOAc) to give ***N*-benzyl-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide (23)** (352 mg, 59% yield). ESMS 393 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 10.0 (s, 1H), 8.21 (d, J = 8.5Hz, 1H), 7.71 (m, 1H), 7.49-7.26 (m, 7H), 4.75 (d, J = 5.5Hz,

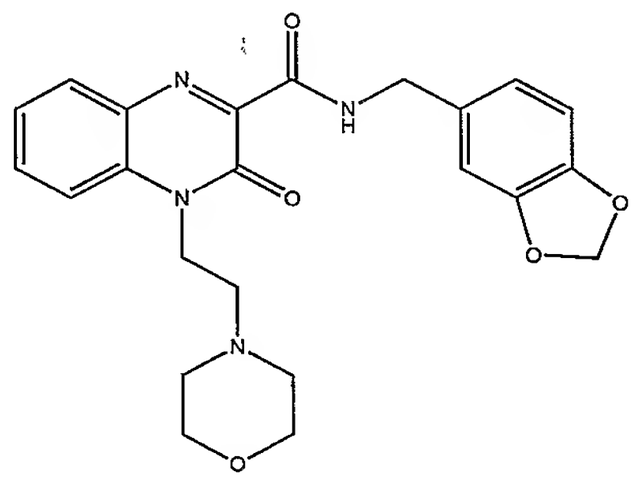
2H), 4.46 (t, J = 6.5Hz, 2H), 3.68 (bt, J = 4.5Hz, 4H), 2.70 (t, J = 7.5Hz, 2H), 2.58 (bs, 4H).

[0229] The following analogs were prepared in a similar fashion:



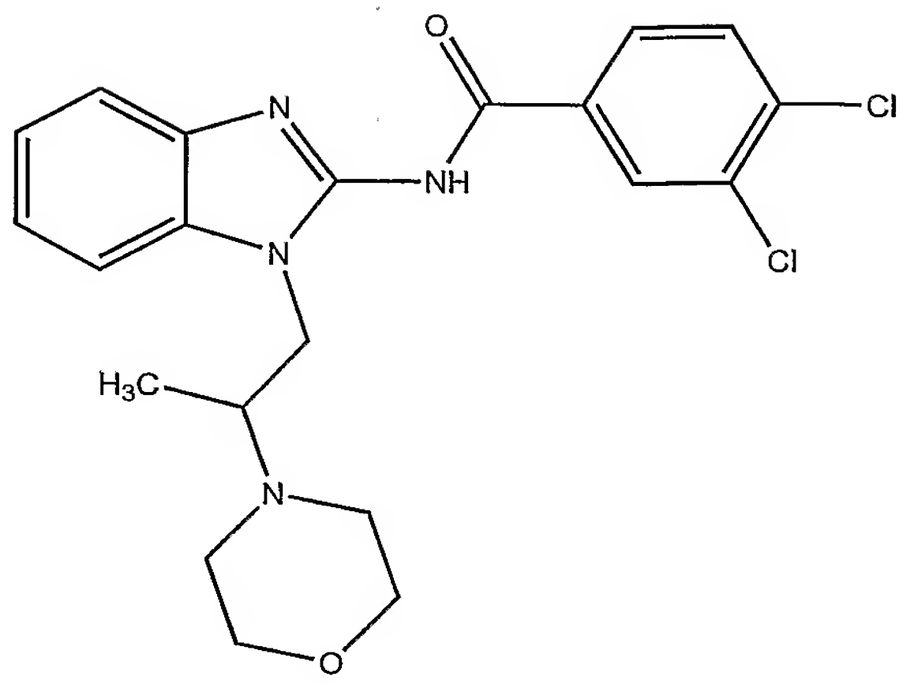
(24)

[0230] **3,4-dihydro-4-(2-morpholinoethyl)-3-oxo-*N*-phenethylquinoxaline-2-carboxamide (24)**, (380 mg, 62% yield) (Rf 0.21, 5%EtOH/EtOAc). ESMS 407 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.71 (s, 1H), 8.19 (d, J = 8.0Hz, 1H), 7.70 (t, J = 7.5Hz, 1H), 7.33-7.20 (m, 7H), 4.46 (t, J = 6.5Hz, 2H), 3.80 (m, 2H), 3.69 (bs, 4H), 2.99 (t, J = 7.5Hz, 2H), 2.58 (bs, 4H).



(25)

[0231] ***N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide (25)** (428 mg, 65% yield) (Rf 0.16, 5%EtOH/EtOAc). ESMS 437 ($M+H^+$). ^{1}Hnmr ($CDCl_3$, 500MHz) δ: 9.95 (s, 1H), 8.21 (d, J = 8.0Hz, 1H), 7.71 (m, 1H), 7.48-7.43 (m, 3H), 6.91 (s, 1H), 6.86 (d, J = 8.0Hz, 1H), 6.76 (d, J = 8.0Hz, 1H), 5.94 (s, 2H), 4.63 (d, J = 5.5Hz, 2H), 4.46 (t, J = 6.5Hz, 2H), 3.68 (bs, 4H), 2.70 (t, J = 6.5Hz, 2H), 2.57 (bs, 4H).



(26)

EXAMPLE 8a

[0232] **3,4-dichloro-*N*-(1-(2-morpholinopropyl)-1*H*-benzo[*d*]imidazol-2-yl)benzamide (26)**. 2-Bromopropanoic acid (3.4 g, 22.37 mmol) was combined with morpholine (7.0 g, 80.46 mmol) and heated overnight at 100°. The remaining morpholine was removed *in vacuo* and the residue taken up in diethyl ether (100 ml). The resultant suspension was filtered and the solvent removed *in vacuo* to give 2-morphlinopropanoic acid. This compound (1.3 g, 8.1 mmol) in dichloromethane (50 ml) at 0° was combined with triethylamine (2.90 g, 101.19, 28.7 mmol, 0.726, 4 ml) followed by 2-nitro aniline (1.23 g, 138, 8.91 mmol), *N*-hydroxysuccinimide (1.0 g, 115.09, 8.91 mmol) and dicyclohexylcarbodiimide (2 g, 206.33, 9.69 mmol) and the reaction mixture allowed to warm to room temperature. After stirring at room temperature over night the reaction mixture was diluted with diethyl ether (50 ml), filtered and the solvent removed *in vacuo*. The residue was purified by chromatography (elution with dichloromethane) to give 2-morpholino-*N*-(2-nitrrophenyl)propanamide. To a solution of this compound (2.1 g, 7.5 mmol) dissolved in ethanol (100 ml) was added 10% Pd/C (50 mg) and the reaction shaken in a hydrogen atmosphere (at 20 pounds per square inch) until consumption of hydrogen had ceased. The reaction mixture was filtered through a pad a filter aid (diatomaceous earth) and the solvent removed *in vacuo* to give *N*-(2-aminophenyl)-2-morpholinopropanamide. To a solution of this

compound (1.0 g, 3.98 mmol) in THF (80 ml) was added borane-THF complex (30 ml, 1.2 M, solution in THF) and the reaction mixture refluxed for 30 hours. Hydrochloric acid (50 ml, 2M) was then added and the reaction mixture refluxed for a further hour. Sodium hydroxide (50 ml, 2M) was then added and the solvent removed *in vacuo*. The residue was extracted with ethyl acetate (3 x 100 ml), the organic phase dried and the solvent removed *in vacuo*. The residue was purified by chromatography (EtOAc) to give N^1-(2-morpholinopropyl)benzene-1,2-diamine. This compound, in a similar manner to that used in the preparation of compound **(2)**, was reacted firstly with cyanogen bromide to give 1-(2-morpholinopropyl)-1H-benzo[*d*]imidazole-2-amine which was then reacted with 3,4-dichlorobenzoyl chloride to give **3,4-dichloro-*N*-(1-(2-morpholinopropyl)-1*H*-benzo[*d*]imidazol-2-yl)benzamide (26).**

EXAMPLE 9

Pharmaceutical Dosage Forms for the Compounds of Formulas I and II

[0233] (1) Injectable Suspension (I.M.) in mg/mL:

Compound of Formula I or II	**10.0**
Methylcellulose	5.0
Tween 80	0.5
Benzyl alcohol	9.0
Benzalkonium chloride	1.0
Water for injection	to a total volume of 1 mL.

[0234] (2) Tablet (in mg/tablet):

Compound of Formula I or II	**25.0**
Microcrystalline Cellulose	415.0
Povidone	14.0
Pregelatinized Starch	43.5
Magnesium Stearate	2.5

[0235] (3) Capsule (mg/capsule)

Compound of Formula I or II	**25.0**
Lactose Powder	573.5
Magnesium Stearate	1.5

[0236] (4) Aerosol (per canister):

Compound of Formula I or II	**24.0**
Lecithin NF Liq. Conc.	1.2 mg
Trichlorofluoromethane NF	4.025 g
Dichlorodifluoromethane NF	12.15 g

EXAMPLE 10

Membrane-based CB_2 binding assay

[0237] The following is a preferred assay using human CB_2-R expressed in HEK293 cells. In round-bottom 96 well plates, test compounds were serially diluted (1:10; 100 µl/well) from 20 µM in assay buffer (50 mM Tris-HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 1 mg/ml BSA; pH 7.5) from stocks (2 mM) prepared in propylene glycol. Five concentrations of each test compound were assayed in a final concentration range of 10^{-5} M to 10^{-9} M. Positive and negative controls (100 µl each, in triplicate) containing no inhibitor are included in each assay plate. To all wells, including those containing the test compound and the positive control, was added [^{3}H]WIN-55,212-2 (Perkin-Elmer Catalog #NET1058; specific activity= 41 Ci/mmol; 24.39 µM) diluted in assay buffer to a concentration of 0.375 nM (80 µl/well – final concentration of 0.15 nM). To the negative control wells is added assay buffer (80 µl). To all wells is added the CB_2 membrane preparation (Perkin-Elmer Catalog #RBXCB2M; 20 µl/well), which has been previously diluted with assay buffer according to the manufacturers instructions. The plates are incubated for 1.5 hours at 37°C, before the membranes are harvested onto glass-fiber mats that had been pre-wetted (3x) with 0.05% poly(ethylene)imine (PEI). The mats were washed (10x) with distilled water and completely dried prior to adding scintillation cocktail (Microscint-O; Perkin-Elmer Catalog #6013611). Bound radioactivity of the plate(s) was measured in a Packard TopCount NXT microplate scintillation counter and the cannabinoid inhibitory activity of the test compound(s) determined (where activity is inversely proportional to the amount of radio-ligand bound). Results were expressed as a percentage of the positive control. IC_{50} values were determined using a graphics software package such as GraphPad Prism 4 (GraphPad, San Diego, Calif.).

[0238] IC_{50} values for CB_2-R binding for the compounds described herein are shown in the Table 4.

TABLE 4

Compound	IC_{50} (μM)
(1)	5.01
(2)	1.0
(3)	2.57
(4)	3.3
(5)	10.5
(6)	25.1
(7)	5.0
(8)	17.8
(9)	3.1
(10)	>100
(11)	>100
(12)	21.4
(13)	3.5
(14)	16.6
(15)	21.4
(16)	5.01
(17)	4.47
(18)	31.6
(19)	1.0
(20)	~25.1
(21)	>100
(22)	>100
(23)	~14.1
(24)	~15.8
(25)	~28.2

EXAMPLE 11

CB_2-Receptor Agonists Inhibit Development of EAE

[0239] EAE is induced and evaluated using the methods and steps described above by administering encephalitogenic T cells which were prepared from spleens isolated from Lewis rats ten days following immunization with MBP in CFA. Four female Lewis rats constitute the control disease group. Groups of 4 animals are each administered the encephalitogenic T cells and treated with the appropriate CB_2-R ligands of Formula I or Formula II twice daily by intraperitoneal injection such that animals receive the agent at the daily dose rates indicated in the table below. Dosing begins on the day of cell transfer.

RESULTS

[0240] All of the animals (4/4) in the control groups were expected to develop clinical disease, although this was not always the case. Depending on the doses, animals in the CB_2-R ligand treated groups may not develop disease, or when they do develop clinical signs, the disease is less severe, with delayed onset and shortened duration versus control animals. Typical results obtained with novel CB_2-R ligands ("drug") based on Formula I and Formula II are summarized in Table 5 below. In this table 'doses' are in mg/kg given twice per day by intraperitoneal injection, where the drug was suspended in pharmaceutical grade olive oil (100 µl per injection). Control animals received vehicle alone. The term 'clin dis' represents the number of animals, out of the total per group, that showed clinical signs at any point during the study. The term 'max dis' is the mean (±SEM) of the maximum clinical disease score for each animal per group. The term 'duration' is mean duration, in days, of apparent clinical signs for each animal in the group.

[0241] Histological examination of the spinal cords of the control and treated animals show the following results. The control group exhibited a heavy lesion burden, on average approximately 15 lesions/section. In contrast, spinal cords from the drug treated group had an average of 1-6 lesions and no more than 10 lesions/section in the most severely affected animal.

TABLE 5

	treatment group					control group		
drug	dose	clin dis	max dis	duration		clin dis	max dis	duration
(1)	10	2/4	0.5±0.3	2.0		4/4	3.5±0.3	3.7
(1)	10	2/4	1.6±0.9	2.5		4/4	2.8±0.7	4.5
(2)	15	3/4	2.7±1.8	3.5		4/4	4.1±0.7	5.5
(2)	15	1/4	0.1	1.0		3/4	2.2±1.4	3.4
(2)	15	0/4	0	0		3/4	1.1±1.4	2.2
(19)	10	4/4	1.4±0.6	2.5		4/4	2.6±0.5	3.8
(19)	10	2/4	1.5±1.2	3.2		4/4	2.6±0.7	4.0
(20)	10	2/4	0.3±0.2	1.6		4/4	1.2±0.3	3.0

EXAMPLE 12

Inhibition of Passively-Transferred Adjuvant Arthritis

[0242] CB_2-R agonists are tested for their ability to suppress the development of adjuvant arthritis in rats that results from the transfer of T lymphocytes isolated from the spleens of adjuvant treated donors. The studies are performed on male DA rats, 8 to 12 week old, using the methods and steps described above. The inducing adjuvant is a suspension of *Mycobacterium butyricum* in mineral oil. Spleens cells from the treated rats are stimulated in culture with Con A for 3 days and injected i.v., into DA recipients (8.5×10^7 cells/rat). Treatment with test agents is initiated on the day of cell transfer. The CB_2-R agonists are administered intraperitoneally at the doses given below. After 5 to 8 days, the hind leg distal joints of saline treated control rats thicken and become hyperemic. Disease severity is evaluated and graded daily as described above.

RESULTS

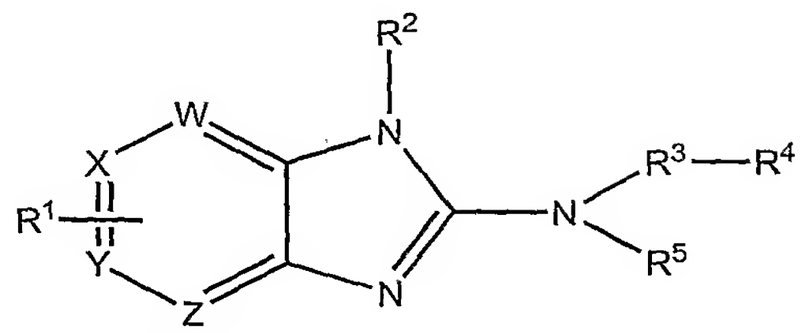
[0243] In a typical example, compound (2) suppressed clinical signs of passively-transferred adjuvant induced arthritis at a dose of 15 mg/kg, intraperitoneally, bid. At the end of a two week treatment period, a highly statistically significant difference in the disease status of the treated versus the control animals was observed. Treated animals showed no inflammation. Specifically 0/4 animals developed swelling ankle joints. In the control group all animals developed clinical signs of disease. The average maximum increase in ankle size seen during the two week experiment was 10.5% (range 3 to 27.3%).

[0244] All the references cited above are incorporated herein by reference in their entirety, whether specifically incorporated or not.

[0245] Having described various embodiments of this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

WHAT IS CLAIMED IS:

1. A compound of Formula I:



Formula I

or a pharmaceutically acceptable salt or prodrug thereof, wherein

(a) R^1 is: H, C_{1-6} alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or C_{2-6} alkoxy;

(b) R^2 is: C_{1-6} alkyl, cycloalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl, wherein n is an integer from 1 to 3;

(c) R^3 is: CHR^6, CO or SO_2;

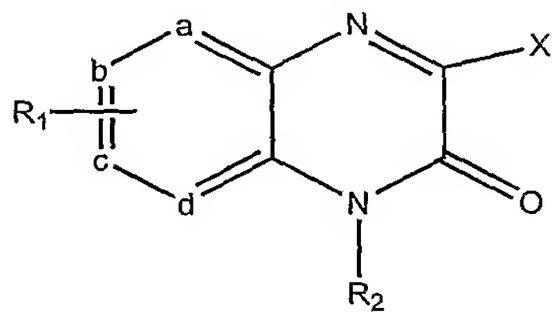
(d) R^4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl;

(e) R^5 is: H or lower alkyl or heteroalkyl;

(f) R^6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy; and

(g) W, X, Y and Z can be either C or N, wherein the total number of nitrogen atoms amongst W, X, Y and Z does not exceed 2.

2. A compound of Formula II



Formula II

or a pharmaceutically acceptable salt or prodrug thereof, wherein

(a) R^1 is: H, C_{1-6} alkyl, halogen, OCH_3, CF_3, OCF_3, $OCHF_2$, OH or C_{2-6} alkoxy;

(b) R^2 is: C_{1-6} alkyl, cycloalkyl, $(CH_2)_n$-heteroalkyl, $(CH_2)_n$-heterocycloalkyl, or $(CH_2)_n$-heteroaryl, wherein n is an integer from 1 to 3;

(c) X is $N(R_3\text{-}R_4)(R_5)$, C(O)Y or C(NH)Y;

(d) R^3 is: CHR_6, CO or SO_2;

(e) R^4 is: lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl or heteroaryl;

(f) R^5 is: H or lower alkyl or heteroalkyl;

(g) R^6 is: H, lower alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl or, carboxy;

(h) Y is: $N(R_3\text{-}R_4)(R_5)$ or C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkynyl, cycloalkyl, heteroaryl, $(CH_2)_n$-heterocycloalkyl, $(CH_2)_n$-aryl or $(CH_2)_n$-heteroaryl, wherein n is an integer of from 1 to 3; and

(i) a, b, c and d can be either C or N, wherein the total number of nitrogen atoms amongst a, b, c and d does not exceed 2.

3. The compound of claim 1 which binds specifically to a cell-bound or cell-free CB_2 receptor with an affinity characterized by a K_d of 100 μM or lower.

4. The compound of claim 2 which binds specifically to a cell-bound or cell-free CB_2 receptor with an affinity characterized by a K_d of 100 μM or lower.

5. The compound of claim 1 which is a CB_2 receptor agonist characterized by its ability to stimulate a CB_2-related post-binding signal transduction event after binding to a CB_2 receptor on a cell.

6. The compound of claim 2 which is a CB_2 receptor agonist characterized by its ability to stimulate a CB_2-related post-binding signal transduction event after binding to a CB_2 receptor on a cell.

7. The compound of claim 5, wherein said signal transduction event is an inhibition of adenylyl cyclase activity and/or induced cAMP generation.

8. The compound of claim 6, wherein said signal transduction event is an inhibition of adenylyl cyclase activity and/or induced cAMP generation.

9. The compound of claim 1 selected from the group consisting of

(a) 4-chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide;

(b) 4-chloro-*N*-[7-methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide;

(c) 3,4-dichloro-N-[1-(2-morpholin-4-ylethyl)-1H-benzimidazo]benzamide; and

(d) 3,4-dichloro-N-(1-(2-morpholinopropyl)-1H-benzo[d]imidazol-2-yl)benzamide.

10. The compound of claim 2 selected from the group consisting of

(a) 2-chloro-N (1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide;

(b) 3-chloro-N-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide;

(c) tert-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate; and

(d) N-benzyl-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide.

11. A pharmaceutical composition comprising

at least one compound of any one of claims l-10; and

at least one pharmaceutically acceptable carrier or excipient.

12. Use of a compound of any of claims 1-10 for treating an inflammatory condition, a cell proliferative disorder or an immune disorder.

13. Use of the pharmaceutical composition of claim 11 for treating an inflammatory condition, a cell proliferative disorder or an immunopathological disorder.

14. Use of a compound of any of claims 1-10 for treating a disease or condition associated with abnormally low activity of, or signalling through, CB_2 receptors of cells of a mammalian immune system.

15. Use of the pharmaceutical composition of claim 11 for treating a disease or condition associated with abnormally low activity of, or signalling through, CB_2 receptors of cells of the immune system.

16. The use of any of claims 12-15, wherein said disease, condition or disorder is selected from the group consisting of graft rejection, graft vs. host disease, T-cell mediated hypersensitivity including T cell-mediated dermatitis, allergic disease, arthritis, preferably rheumatoid arthritis, insulin-dependent diabetes mellitus, multiple sclerosis, acute disseminated encephalomyelitis, asthma, chronic obstructive pulmonary disease, emphysema, bronchitis, acute respiratory distress syndrome, inflammatory bowel disease such as Crohn's disease, lupus, ischemic or reperfusion injury, celiac disease, atopic dermatitis. psoriasis, urticaria, scleroderma, mycosis fungoides, dermatomyositis, alopecia areata, chronic actinic dermatitis, stromal keratitis, eczema, Behcet's disease, Pustulosis palmoplanteris, Pyoderma gangrenum, Sezary's syndrome, systemic sclerosis, morphea, autoimmune thyroid disease, Addison's disease, an autoimmune polyglandular disease or syndrome, sialitis and Sjögren's syndrome, pernicious anemia, vitiligo, Guillain-Barre syndrome, glomerulonephritis and serum sickness.

17. The use according to any one of claims 14-16, wherein said compound is administered orally, parenterally or topically.

18. The use according to any of claims 14-16, wherein said compound is administered in combination with an agent that is also useful for the treatment of symptoms or underlying cause of said disease or condition.

19. The use of claim 18, wherein said agent is selected from the group consisting of methotrexate, sulfasalazine, a COX-2 inhibitor, hydroxy chloroquine, cyclosporine A, D-penicillamine, infliximab, etanercept , auranofin, aurothioglucose, sulfasalazine, sulfasalazine analogs, mesalamine, corticosteroids,

corticosteroid analogs, 6-mercaptopurine, cyclosporine A, methotrexate and infliximab, interferon β-1β, interferon β-1α, azathioprine, glatiramer acetate, a glucocorticoid and cyclophosphamide.

20. A method for modulating (a) CB_2 receptors on a cell or (b) signal transduction mediated by CB_2 stimulation of a cell, comprising contacting the cell with a receptor-modulatory amount of the compound of any of claims 1-10.

21. The method of claim 20, wherein said compound is one that activates CB_2 receptors.

22. The method of claim 20, wherein said compound is one that blocks the activation of said CB_2 receptors by an endogenous or exogenous CB_2 agonist.

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(54) Title: HETEROCYCLIC CANNABINOID CB2 RECEPTOR ANTAGONISTS

(57) Abstract: Novel selective cannabinoid CB_2 receptor ligands, primarily agonists, have a number of biological and pharmacological activities, including bronchial action, immunomodulatory action and analgesia. Hence, they are useful for treating diseases or conditions characterized by pain, inflammation and immunological dysregulation. Examples of this class of novel compounds are 4 chloro-*N*-[1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide and 4-chloro-*N*-[7 methoxy-1-(2-morpholin-4-ylethyl)-1*H*-benzimidazol-2-yl]benzamide. Other examples of the novel compounds are 4-derivatives of 3-amino and 3-carboxy-1,2-dihydro-1-substitutedquinazol-2-ones, including 2-chloro-N (1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide, 3-chloro-N-(1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-yl)benzamide, tert-butyl 1,2-dihydro-1-(2-morpholinoethyl)-2-oxoquinoxalin-3-ylcarbamate and N-benzyl-3,4-dihydro-4-(2-morpholinoethyl)-3-oxoquinoxaline-2-carboxamide. The compounds bind specifically to a cell-bound or cell-free CB_2 receptor with an affinity (K_d) ≤ 100 μM. As agonists, these compounds stimulate a CB_2-related post-binding signal transduction event, e.g., inhibition of adenylyl cyclase activity, after binding to a CB_2 receptor on a cell. These compounds are used to treat inflammatory conditions, cell proliferative disorders, or an immune disorder, and may be administered in combination with agents that are also useful for the treatment of the symptoms or cause of the underlying disease or condition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/027809

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D471/04 C07D235/30 C07D241/44 A61K31/4184 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/085866 A (WALPOLE CHRISTOPHER ; CHENG YUN-XING (CA); YANG HUA (CA); TOMASZEWSKI) 31 October 2002 (2002-10-31) page 1, line 4 - line 8; claim 1	1-22
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X	US 4 002 623 A (KADIN SAUL BERNARD) 11 January 1977 (1977-01-11) claim 1	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
25 February 2005	14. 03. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seelmann, I

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/027809

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/00611 A (JANSSENS FRANS EDUARD ; SOMMEN FRANCOIS MARIA (BE); ANDRIES KOENRAAD J) 4 January 2001 (2001-01-04) claim 1; tables 13,14; compounds 284,285,286	1
X	WO 00/18767 A (HE XIAO SHU ; NEUROGEN CORP (US)) 6 April 2000 (2000-04-06) claim 1; compound 1	1
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X	ANISIMOVA, V. A.; ET AL.: "SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 1-N- AND 10-N-SUBSTITUTED 1(10),2,3,4-TETRAHYDROPYRIMIDO-‘1,2-a!BENZ IMIDAZOLES" PHARMACEUTICAL CHEMISTRY JOURNAL, vol. 36, no. 9, 2002, pages 468-473, XP002308511 compounds IVE, VIA-D, VIIA-D	1
X	EP 1 048 655 A (NIHON NOHYAKU CO LTD ; TAISHO PHARMA CO LTD (JP)) 2 November 2000 (2000-11-02) claim 1; compound 27	2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/027809

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1,3,5,7,9,11-22(part)

condensed imidazoles

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2. claims: 2,4,6,8,10,11-22(part)

condensed pyrazines

INTERNATIONAL SEARCH REPORT

ɪrmation on patent family members

Inter[redacted]nal Application No
PCT/US2004/027809

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